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AGRICULTURAL RESEARCH INSTITUTE

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THE JOURNAL OF AGRICULTURAL SCIENCE

LONDON
Cambridge University Press
FETTER LANE, E.C. 4

CHICAGO
The University of Chicago Press
(Agents for the United States)

BOMBAY · CALCUTTA · MADRAS
Macmillan

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THE JOURNAL OF AGRICULTURAL SCIENCE

EDITED FOR THE PLANT BREEDING AND ANIMAL NUTRITION RESEARCH INSTITUTES AT CAMBRIDGE,
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E. M. CROWTHER, D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden
SIR A. D. HALL, K.C.B., M.A., LL.D., F.R.S., John Innes Horticultural Institution, Merton Park, London, S.W. 19
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SIR E. J. RUSSELL, D.Sc., F.R.S., Rothamsted Experimental Station, Harpenden

IN CONSULTATION WITH

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DR H. J. WHEELER, American Agricultural Chemical Co., Agricultural Service Bureau, 419 Fourth Avenue, New York, U.S.A.

VOLUME XXV 1935

CAMBRIDGE
AT THE UNIVERSITY PRESS
1935

PRINTED IN GREAT BRITAIN

CONTENTS

PART 1 (JANUARY 1935)

PAGE

ELPHICK, B. L. and McMAHON, P. R. Note on the estimation of dry matter in mangels 1

SREENIVASAN, A. and SUBRAHMANYAN, V. Biochemistry of water-logged soils. Part IV. Carbon and nitrogen transformations. (With six text-figures) 6

DUNLOP, GEORGE. The calcium, phosphorus and vitamin D requirements of swine. (With one text-figure) . . 22

WOODMAN, H. E. and EDEN, A. Nutritive value of lucerne. III. The composition, digestibility and nutritive value of lucerne hay, lucerne meal (English and American) and lucerne leaf meal (American) 50

GOOCH, MARJORIE. An analysis of the time change in milk production in individual lactations. (With five text-figures) 71

MARSTON, HEDLEY R. Studies on the relationship between nutrition and wool production of Merino sheep. I. The technique employed for determining the utilisation of foodstuffs and for estimating the wool produced over short periods by Merino sheep. (With three text-figures) . . 103

MARSTON, HEDLEY R. Studies on the relationship between nutrition and wool production of Merino sheep. II. The effect of the administration of cystine, cystein, sulphur and methionine on the growth of wool of a Merino ewe on a protein-poor ration 113

RICHARDSON, H. L. and CROWTHER, E. M. Studies on calcium cyanamide. V. The utilisation of calcium cyanamide in pot culture experiments. (With three text-figures) . . 132

DUNLOP, GEORGE. The control of variation in gain in animal nutrition experiments. (With one text-figure) . . 151

PART 2 (APRIL 1935)

PAGE

ARMSTRONG, J. M. and WHITE, W. J. Factors influencing seed-setting in alfalfa. (With Plate I and five text-figures) . 161

DEIGHTON, THOMAS. A study of the fasting metabolism of various breeds of pig. II. Body temperature measurements. (With three text-figures) 180

DOYNE, H. C. Studies in tropical soils. Increase of acidity with depth 192

HAVAS, LÁSZLÓ. Some effects of radioactive mud upon germination of seeds and growth of seedlings. (With Plates II and III and six text-figures) 198

DUNLOP, GEORGE. The vitamin A requirement of swine. (With one text-figure) 217

CHRISTIDIS, BASIL G. Intervarietal competition in yield trials with cotton. (With one text-figure) 231

BARTLETT, M. S. An examination of the value covariance in dairy cow nutrition experiments 238

BELL, G. D. H. Preliminary experiments on vernalisation. (With four text-figures) 245

HENDRICKS, WALTER A. The use of "differential regression" in analysis of variance 258

HADFIELD, J. W. and CALDER, R. A. A genetic analysis of the seed characters wrinkled, dimpled and smooth in *Pisum*. (With Plates IV and V) 264

VIRTANEN, ARTTURI I. and VON HAUSEN, SYNNÖVE. Investigations on the root nodule bacteria of leguminous plants. XVI. Effect of air content of the medium on the function of the nodule and on the excretion of nitrogen . . 278

VIRTANEN, ARTTURI I. and VON HAUSEN, SYNNÖVE. Investigations on the root nodule bacteria of leguminous plants. XVII. Efficiency of different strains of clover nodule bacteria 290

BATES, G. H. A study of the factors influencing size of potato tubers. (With one text-figure) 297

Part 3 (July 1935)

PAGE

Garner, F. H., Grantham, J. and Sanders, H. G. An electric oven for drying samples from field plots. (With Plate VI) 315

Romanoff, Alexis L. Influence of incubation temperature on the hatchability of eggs, post-natal growth and survival of turkeys. (With four text-figures) 318

Rogers, W. S. A soil moisture meter. Depending on the "capillary pull" of the soil. With illustrations of its use in fallow land, grass orchard, and irrigated orchards. (With Plate VII and seven text-figures). 326

Galpin, Nancy. The pre-natal development of the coat of the New Zealand Romney lamb. (With ten text-figures) . 344

Garner, F. H. and Sanders, H. G. Investigations in crop husbandry. II. On the age of seed beans 361

Paterson, D. D. The growth, yield and composition of certain tropical fodders. (With three text-figures) 369

Reimers, J. H. W. Th. and Bartel, L. H. The retention of protein by growing pigs 397

Hunter, H. The improvement of winter oats 419

Dunlop, George. The effect of the growth promoting, appetite-stimulating or "physin" factor on the live-weight increase of swine 445

Part 4 (October 1935)

Frankel, O. H. The differentiation of grain samples of closely related varieties of wheat by means of a simple mechanical test for grain quality 461

Frankel, O. H. Analytical yield investigations on New Zealand wheat. II. Five years' analytical variety trials. (With one text-figure) 466

PAGE

COCHRAN, W. G. A note on the influence of rainfall on the yield of cereals in relation to manurial treatment. (With one text-figure) 510

COUTTS, J. R. H. "Single value" soil properties: A study of the significance of certain soil constants. VIII. Relationships between the sticky point and the nature of the exchangeable ions in the soil. (With two text-figures) . 523

NORMAN, A. G. The composition of crude fibre 529

HIRZEL, R. Note on the effect of "condition" on the colour of body fat in rabbits 541

YATES, F. and ZACOPANAY, I. The estimation of the efficiency of sampling, with special reference to sampling for yield in cereal experiments. (With two text-figures) . . 545

WOODMAN, H. E. and EVANS, R. E. Nutritive value of lucerne. IV. The leaf-stem ratio 578

WALKLEY, ALLAN. An examination of methods for determining organic carbon and nitrogen in soils. (With one text-figure) 598

HARDY, F., McDONALD, J. A. and RODRIGUEZ, G. Leaf analysis as a means of diagnosing nutrient requirements of tropical orchard crops 610

DILLON WESTON, W. A. R. and BOOER, J. R. Seed disinfection. I. An outline of an investigation on disinfectant dusts containing mercury. (With plate VIII and four text-figures) 628

VOLUME XXV JANUARY, 1935 PART 1

NOTE ON THE ESTIMATION OF DRY MATTER IN MANGELS.

BY B. L. ELPHICK AND P. R. McMAHON.
(Massey Agricultural College, Palmerston North, New Zealand.)

INTRODUCTION.

IN the course of an investigation into the dry-matter yields per acre of different varieties of mangels it became apparent that the method of root sampling recommended by Wood and Berry (1), namely the taking of a core through the largest diameter of the root, did not give sufficiently representative samples of the roots examined. A survey was therefore made of some of the methods of root sampling which had at various times been employed. They are:

(1) The removal of a finely divided sample by bisecting the root longitudinally with a coarse-toothed saw.
(2) The removal of a wedge-shaped sector from the crown to the tip of the root.
(3) The removal of a sample by means of an auger.
(4) The removal of a core from the root by means of a special sampler.

Apart from true representation, such factors as ease and rapidity of sampling, convenience of storage of samples during transport to the laboratory, and the avoidance of changes in the sample due to oxidation and evaporation have all to be borne in mind. Again, any method which yields the sample in a smashed condition seems undesirable, save where analysis can be commenced immediately, while in an ideal method the continued growth and seeding of the plant would not be impaired.

Consideration of these points suggested that the coring method had many advantages over the others, and this paper records an investigation of the accuracy of the method when applied to the examination of the globe type of mangel, of which the variety Sutton's Prizewinner Yellow Globe has been taken as representative.

The immediate object has therefore been to determine the degree of representation afforded by cores taken through different portions of the root. Save for Davey's comprehensive work on sampling of swedes, little information on this point is available in the literature on the subject and generally no description of the sampling method is given.

According to Wood and Berry (1), the water content of a core taken through the largest diameter agrees closely with the mean water content of the whole root, while a core taken diagonally is likely to contain too great a proportion of the central more watery material, especially in the case of the long varieties. The former opinion is contrary to our own experience.

Collins (3), working on sugar estimation in swedes, states that for rough purposes a single boring gives a true sample, the direction and position being unimportant.

Fruwirth (2) discusses several coring methods of sampling and re[illegible]mends oblique cores either through the shoulder or the lower part o[illegible] root and, for some purposes, a short vertical boring up the centre of the root from the lower end.

Sansome (4), working on swedes, gives reasons for the selection of a horizontal north-south core passing through the vertical axis of the root.

Lauder (5) recommends sampling swedes by removing a core passing diagonally from the shoulder of the root.

Davey (6), in a recent paper, gives full details of a critical examination of the core method of sampling when applied to swedes.

TECHNIQUE.

Absolute determinations of dry matter in vegetable materials are not possible by oven drying owing to chemical changes. The method recommended by Lauder (5) proved satisfactory for comparative purposes, and was used throughout this work. Minced material from whole roots and thin slices from individual cores were dried for 40–48 hours in an electric ventilated oven at about 80° C. The dehydration was considered satisfactory if the change on drying for a further period of 1 hour was less than 0·01 gm. on a 50 gm. sample. Duplicates agreed to 0·2–0·3 per cent. of dry matter. It was found that more consistent results were obtained when the material to be dried was spread to a depth of not more than $\frac{1}{2}$ in. in a flat-bottomed dish, than when it rested to a greater depth in a round-bottomed dish. In the former case a much greater proportion of moisture was lost during the first 24 hours (99 per cent. as compared with 80–90 per cent.). When drying took place so slowly considerable chemical decomposition occurred and the values obtained for the dry-matter content were 5–10 per cent. lower than with more rapid drying. Oven drying was compared with the xylene distillation method with the following results:

Sample...	*A*	*B*
	%	%
Oven drying	14·4	14·7
Xylene distillation	14·6	14·7

It was concluded that the loss on drying represents the actual water content with a sufficient degree of accuracy.

A special sampler was constructed from thin-walled ½ in. nickelled brass tubing.

EXPERIMENTAL RESULTS.

On account of the fact that mature roots unaffected by changes following lifting are available for only a short time each year, the amount of work that could be done in any one season was limited. The following work was extended over the 1930, 1931 and 1932 seasons, and it should be mentioned that there were considerable differences in the soils on which these three crops were grown, and also in the seasons themselves.

The roots were sampled when they were considered ready for lifting but before they had been moved. No obviously abnormal roots, either as regards shape or size, were examined.

1930 *season*. In the first examination each of five roots was sampled by taking a core one-quarter to one-third way down from the crown running horizontally through the axis of the root in a north-south direction. This method was adopted as tentative after a study of the diagram given by Wood and Berry showing the distribution of dry matter within the root.

The mean dry-matter percentages obtained were:

	%
Cores	11·3.
Whole root	12·4

1931 *season*. In the second examination three horizontal cores in the direction north-south and passing through the vertical axis, were taken from each of sixteen roots in the following positions:

	Mean dry-matter %
Core A. About midway between the largest diameter and the crown of the root	9·6
Core B. Through the largest diameter	9·4
Core C. Equidistant from *B* and the bottom of the bulb	9·9
Whole roots	9·8

In view of the irregular shape of most roots it is obvious that these positions are only approximate and will vary slightly for each root.

Comparing the dry-matter percentage over the whole root with that of cores in the *A* position, the standard deviation of the difference of a single pair of observations is 0·72, while the corresponding figures for the *B* and *C* positions are 0·46 and 0·40 respectively.

The results indicate that for these roots there was no significant difference between the percentage of dry matter in the cores in the *A* and *C* positions and in the whole root.

The difference between the result for the *A* position and that obtained the previous year is striking, and may possibly be explained by the fact that the *A* position in 1931 was slightly higher than the corresponding position in 1930, so that the 1931 *A* cores contained a smaller proportion of the inner and moister part of the root and were also more likely to encounter air spaces beneath the crown, both factors leading to a higher proportion of dry matter in the core.

If this surmise be correct, the exact position of the core in the upper part of the root would appear to be critical in its effect on the representative nature of the sample, making it unsafe to rely on a core from this zone. For this reason cores from the *A* position were not taken in further work.

1932 *season*. In this season's work only two cores were taken from each root, as follows:

	Mean dry-matter %
Core B. Through the largest diameter of the root	10·1
Core D. About one-third to one-half way from the largest diameter to the bottom of the bulb	10·9
Whole root	10·7

Comparing the dry-matter percentage over the whole root with that of cores in the *B* position, the standard deviation of the difference of a single pair of observations was 0·382, while the corresponding figure for the *D* position was 0·538.

In these roots there was no significant difference between the percentage of dry matter in the cores in the *D* position and in the whole root.

SUMMARY.

The results indicate that the usually accepted methods of sampling mangel roots by means of horizontal cores in a north-south direction through the largest diameter of the root or one-third way down from the rown, are subject to greater inaccuracies than similar cores taken two-hirds to three-quarters way down, which gave consistently samples of atisfactory accuracy.

Our best thanks are due to members of the College Staff for helpful riticism of all stages of the work, also to the Edinburgh and East of cotland College of Agriculture for a detailed description of technique nd apparatus in use for their work on dry matter in root crops.

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(*Received July 27th*, 1934.)

BIOCHEMISTRY OF WATER-LOGGED SOILS.

PART IV. CARBON AND NITROGEN TRANSFORMATIONS.

By A. SREENIVASAN and V. SUBRAHMANYAN.

(*Department of Biochemistry, Indian Institute of Science, Bangalore.*)

(With Six Text-figures.)

Although the study of carbon and nitrogen changes in dry cultivated soils has drawn the attention of a number of investigators, yet the literature relating to similar transformations in wet cultivated soils is comparatively limited. Itano and Arakawa (11) studied the effect of application of organic materials of widely different carbon-nitrogen ratios to rice fields in Japan and found that (*a*) there was no definite correlation between C/N ratio and crop production, and (*b*) the ratios finally attained were lower than that of the original soil. Osugi, Yoshie and Komatsubara (17) observed that the transformation of organic matter under paddy field conditions is slower than in dry soils and is largely determined by the presence of available forms of carbon and nitrogen.

Some useful information is available, however, regarding the effect of application of nitrogenous fertilisers to swamp soils. The earlier work on this subject has already been considered (19-21). Among the more recent investigations, mention should be made of the work of Janssen and Metzger (12) who observed that in flooded soils nitrates are rapidly lost, while there is steady increase in ammonia. Bartholomew (3) found organic forms of nitrogen to be most suitable for the rice crop, though loss by nitrification and consequent leaching has to be feared. He observed that denitrification also proceeds so rapidly that only small quantities of nitrite can be detected at any time in the soil. Joachim and Kandiah (13) noted that when green manures are ploughed under immediately before flooding, large quantities of ammonia are formed. On the other hand, if the manure is turned under semi-dry soil several weeks before transplanting, nitrates are formed in large quantities and are lost when the land is flooded.

The significance of many of the biochemical changes that attend the decomposition of organic matter in swamp soils is still obscure. Harrison and Aiyer (1, 6-10) adduced evidence to show that the gases, such as methane, hydrogen and carbon dioxide, which are first formed, are oxidised and decarbonised and thus rendered innocuous by the combined action of certain bacteria and algae present at the surface of the soil. On the other hand, Onodera (16) finds that the gaseous products are inimical to the growth of the rice plant, both on account of their direct toxic action and by exclusion of the necessary supply of oxygen. The direct as well as indirect effects of organic acids, such as lactic, acetic and butyric, which are formed in considerable quantities during the decomposition of different organic materials (Subrahmanyan (19–21); Desikachar, unpublished data) on plant growth have not so far been defined. The economy of carbon and nitrogen at different stages during the fermentation has also yet to be studied. These and allied problems are under investigation in these laboratories. The present communication relates to certain aspects of the carbon and nitrogen transformations attendant on the application of different organic substances to water-logged soils.

EXPERIMENTAL.

Preliminary. A local paddy soil was collected shortly after harvest, air-dried, ground to pass the 3 mm. sieve, and weighed out in 100 gm. lots into a number of 250 c.c. conical flasks. They were treated with varying quantities of organic substances with different C/N ratios (Table I) in the form of powders passing a 1 mm. mesh sieve. Water was added at the rate of 100 c.c. per flask; this quantity was found sufficient to keep the soil well submerged. The flasks were plugged with cotton-wool and incubated at 30° C. The contents of the flasks were examined at frequent intervals for (1) ammonia present in the supernatant liquid and in the soil sediment, (2) nitrite, and (3) nitrate using colorimetric methods (14).

There was fairly vigorous fermentation in all cases (except those of urea and cyanamide) accompanied by evolution of carbon dioxide. Only traces of ammonia were generally present in the supernatant liquid, while the major part remained associated with the soil sediment and could be extracted only by repeated leaching with salt solution.

In the early stages, only traces of nitrites and nitrates were present, though later increasing quantities of nitrates were formed. It was also noted that when the different materials were used in quantities containing less than about 30 mg. of nitrogen, the products of decomposition were formed in such minute amounts that they could not be accurately estimated. The transformations of nitrogen were also generally slow (except in the cases of urea, cyanamide and dried blood), so that it was found convenient to follow them at weekly intervals.

The carbon contents of the different materials were determined according to Degtjareff (4). As observed by Walkley and Black (22), this method was found to give exaggerated values, so some parallel determinations were carried out comparing the results with those obtained by a modified method of wet combustion developed in these laboratories (unpublished). The main features of the method consist in oxidising the organic matter with chromic acid followed by displacement of the resulting CO_2 by steam. The vapours are cooled and the CO_2 absorbed in the condenser which is packed with glass beads over which a gentle stream of alkali is kept flowing. Thus, it was observed that the carbon content of the soil, as estimated by Degtjareff's method, was 0·34 per cent., while the value obtained by combustion was 0·23 per cent. Total nitrogen was estimated according to the wet digestion method of Sreenivasan and Subrahmanyan (18). The results have been presented in Table I.

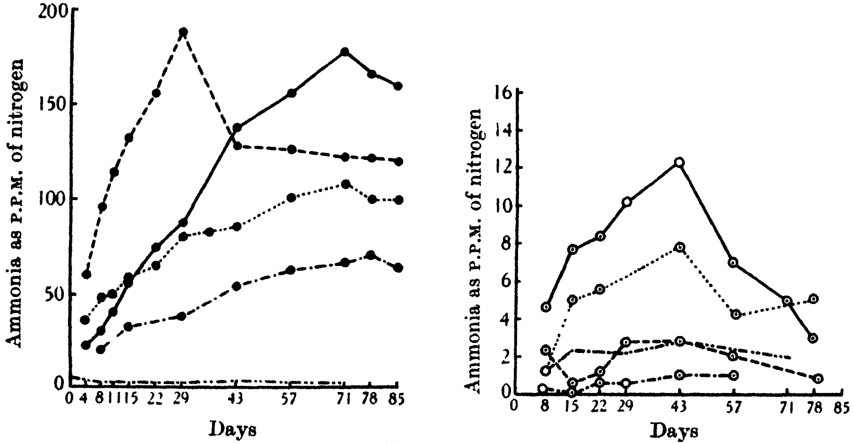
Table I.

Substance	Percentages Carbon	 Nitrogen	C/N
Cyanamide	15·9	17·6	0·9
Dried blood	41·1	12·5	3·3
Urea	20·0	46·7	0·4
Hongay (*Pongamia glabra*) leaf	47·0	3·4	13·8
Farmyard manure	5·9	0·6	9·8
Mahua (*Bassia latifolia*) cake	40·4	2·7	14·9
Lantana (*Lantana camara*) (whole plant)	43·0	1·9	22·6
Rice straw	36·1	0·3	120·3
Soil	0·23	0·023	10·0

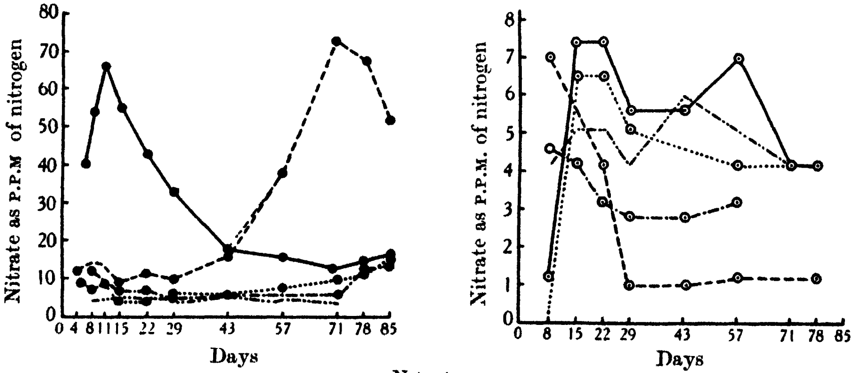
Quantitative studies. To 100 gm. lots of soil, the different substances were added in quantities corresponding to 30 mg. of combined nitrogen. The mixtures were treated with 100 c.c. each of water and the aqueous suspensions incubated at 30° C. Losses of water through evaporation were made up from time to time by fresh additions. The contents of the flasks were generally analysed at weekly intervals, but in the cases of cyanamide, dried blood and urea, where the decomposition was very rapid during the first few weeks, the specimens were examined twice a week. Ammonia and nitrate were estimated according to Olsen (15). The values for nitrate were also checked independently by the phenol disulphonic acid method (14). Nitrite was estimated by the Griess-Ilosvay method (14). Total carbon and nitrogen were estimated according to methods already cited. H-ion concentration was determined colorimetrically using a Hellige comparator.

H-*ion concentration.* In most cases the reaction remained practically the same (*p*H 7·4–7·6) throughout the period of observation. With mahua cake and rice straw, however, the medium became slightly acid (pH 5·7–5·9) within the first two weeks, after which it rose steadily to pH 7·6.

Ammonification (Fig. 1). Production of ammonia was most rapid and complete with cyanamide and urea, 60 per cent. of the nitrogen being ammonified.



Ammonia



Nitrates

Fig. 1. Production of ammonia and nitrates.

●——● Cyanamide ●····● Dried blood ⊙——⊙ Farmyard manure
⊙····⊙ Mahua cake ●– –● Urea ●–·–● Hongay leaf
—··—·· Control ⊙– –⊙ Lantana ⊙–·–⊙ Rice straw

Next in order came dried blood (37 per cent.) and hongay leaf (23 per cent.), while farmyard manure, mahua cake, lantana and straw showed poor ammonification, hardly 5 per cent. of the added nitrogen being converted into that form. The slowness of fermentation with mahua may be explained by the presence of the toxic saponin, though the C-N ratio was otherwise favourable to the decomposition. The essential oil of lantana may also have interfered with ammonification. In lantana and straw there was a large excess of carbon over nitrogen, and the mineralisation of the latter was greatly retarded. The farmyard manure used in the present study was of inferior quality, having been diluted with a large quantity of powdered soil and ash and having apparently lost a large part of its nitrogen through indifferent storage.

Except in the case of urea, the maximum production of ammonia was generally between the 71st and 78th days. After that period the quantities tended to diminish.

Nitrification (Fig. 1). As distinct from the usual observations on dry soils, nitrification was generally slow. In the early stages, when the fermentation was most vigorous, practically no nitrate was formed; the quantities produced in the later stages were small and bore no close relation to those of ammonia present in the medium. In the case of urea, the rapid ammonification was succeeded (after the 29th day) by nitrification, and increasing quantities of nitrate were formed up to the 71st day: after that period the quantities tended to diminish. Dried blood and hongay leaf showed comparatively poor nitrification. The quantities of nitrate formed from farmyard manure and mahua cake were very small, while those present in soils treated with lantana or straw were even less than those in the untreated soil. In contrast to the above, there was fairly rapid formation of nitrate from cyanamide almost from the commencement. The quantities continued to increase during the first eleven days, after which they decreased.

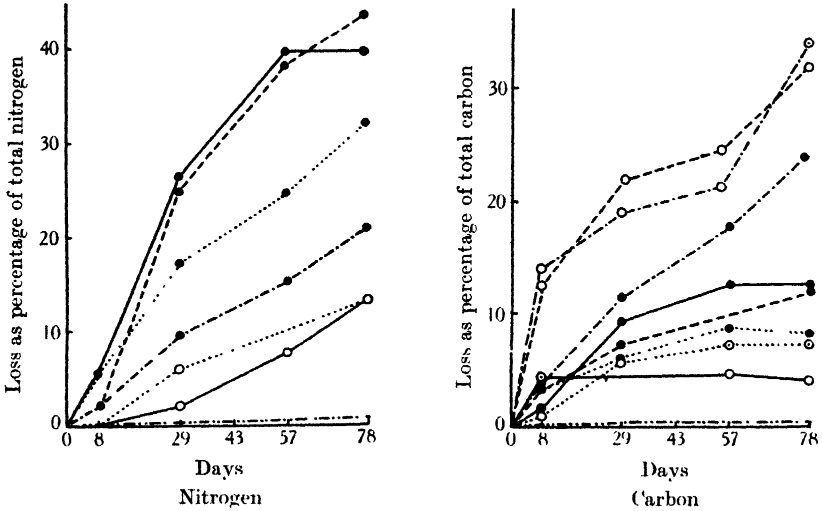
The conditions relating to nitrification in the swamp soil are still obscure, but there is evidence to suggest that the formation of nitrate is possible only when the decomposition of the added organic matter is fairly well advanced and the dissolved oxygen content of the soil system is improved.

Table II. *Total mineralised nitrogen (as parts per million).*

	Days												Maximum recovery of added nitrogen (as %)
Treatment	4	8	11	15	22	29	43	57	71	78	85	170	
Cyanamide	60	84	106	111	117	122	156	172	191	181	177	145	64
Dried blood	46	55	59	61	69	85	101	109	118	112	115	77	39
Urea	72	109	126	140	166	197	155	164	195	189	173	175	65
Hongay leaf	—	32	—	40	45	59	67	73	76	80	79	50	27
Farmyard manure	—	6	—	15	16	16	18	14	9	7	—	8	6
Mahua cake	—	1	—	11	12	11	13	8	—	9	—	20	4
Lantana	—	9	—	8	5	4	4	3	—	2	—	6	3
Straw	—	5	—	4	4	3	4	4	—	—	—	5	2
Untreated (control)	—	5	—	7	7	7	9	5	6	—	—	4	—

Mineralisation of nitrogen. The quantities of mineralised nitrogen present at different stages and the maximum recovery of added nitrogen are given for each treatment in Table II.

Taken as a whole, the results suggest that the chief factor determining the efficiency of mineralisation of nitrogen is the ratio of carbon to nitrogen, those with narrow ratios mineralising very much more rapidly than those with wide ones. Although the above observations correspond to those obtaining in dry soils, it may yet be noted that whereas in the swamp soil ammonia is the chief form of mineralised nitrogen, in the dry soil it is mostly nitrate.



Nitrogen

Carbon

Fig. 2. Loss of carbon and nitrogen during decomposition.

●——● Cyanamide
⊙---⊙ Mahua cake
—··—·· Control
●····● Dried blood
●– – –● Urea
○—○ Lantana
⊙——⊙ Farmyard manure
●–·–● Hongay leaf
○---○ Rice straw

Loss of carbon and nitrogen (Fig. 2). With substances of narrow C/N ratio, such as cyanamide, urea or dried blood, the loss of carbon even at the end of 170 days was negligible and no greater than that in the control. Hongay leaf and farmyard manure lost the major part of their carbon during the period of observation. The losses from lantana and straw correspond to one-third of the total carbon: even then the equilibrium stage had not been attained, so that it would appear that from substances with wide C/N ratios, the loss of carbon, though steady, is comparatively slow.

The losses of nitrogen went in the opposite direction. Cyanamide, urea and dried blood suffered the maximum loss (over 60 per cent.). Hongay leaf and farmyard manure came next with about 40 per cent. each, while mahua cake and lantana lost about 20 per cent. each in the course of 170 days. The loss of nitrogen from rice straw was almost negligible and was not significantly different from that in the control.

It may be noted that even with substances of narrow C/N ratio there is very little loss of nitrogen during the first few weeks, when the fermentation is most vigorous. This is followed, however, by rapid loss which continues up to the 78th day. Although some nitrate is formed during the latter period, it is relatively small as compared with the quantities of ammonia present in the medium, and it would appear that the loss of nitrogen is in some way associated with the tardy nitrification.

Changes in C/N *ratio.* It may be seen from Table III that there is a general tendency to attain equilibrium in the region of 11 to 1, which is about that of the untreated soil. As would be expected, substances with narrow C/N ratios lose a part of their nitrogen and those with wide ratios a part of their carbon to attain that equilibrium. On the other hand, substances like hongay leaf and farmyard manure which start with very nearly the optimum ratio lose both carbon and nitrogen in such a manner that the ratio of the residue is not very much altered. Mahua cake is, however, an exception to the above: it undergoes practically no decomposition during the period of observation and its ratio remains unaltered.

Table III. *Carbon-nitrogen ratios.*

	Days					
Treatment	0	8	29	57	78	170
Cyanamide	6·9	7·2	8·5	10·0	10·0	10·7
Dried blood	8·3	8·4	9·3	10·0	11·2	10·6
Urea	6·6	6·5	8·1	8·9	10·3	11·5
Hongay leaf	13·2	13·9	13·9	13·8	13·6	11·7
Farmyard manure	11·6	10·9	11·4	11·7	13·0	10·3
Mahua cake	14·9	14·5	14·9	16·0	15·0	15·0
Lantana	19·4	16·6	14·7	15·0	13·4	14·4
Rice straw	69·0	59·4	53·9	53·4	46·6	45·8
Untreated	10·0	10·0	9·8	—	10·3	10·9

Distribution of nitrogen. The quantities of nitrogen taken up by micro-organisms or otherwise present as complex forms were calculated from the difference between the total and mineralised nitrogen present at each stage.

It was noted that in the case of substances with narrow C/N ratios, only a small portion of the nitrogen was held as complex forms; the major part was either mineralised and present as soluble forms or lost altogether from the soil system. This would suggest that unless the crop is in a position to take up the bulk of the available nitrogen, the added material would be largely wasted. On the other hand, substances with wide ratios do not mineralise to any appreciable extent, so that they are of no immediate use to the crop. Practically all their nitrogen is, however, present in complex form and may prove available to a subsequent crop. It may thus be seen that materials with extreme C/N ratios would not be of much practical utility: only those of intermediary character (like the hongay leaf), which mineralise at a moderate rate and, at the same time, retain a useful part of their nitrogen as complex forms, would appear to be suitable in field practice.

Influence of sulphur or lime on the mineralisation of nitrogen. Bal and Misra (2) observed that the growth of the rice plant is favoured by neutral or slightly acid reaction. With a view to determining whether the addition of a substance known to render the soil acid facilitates increased mineralisation of nitrogen, some experiments were carried out adding 50 mg. of sulphur to 100 gm. lots of soil, treated with the different organic substances in the manner already described. Parallel experiments were also carried out adding burnt lime (50 mg.) in place of sulphur.

It may be observed from Fig. 3 that the addition of sulphur had a slightly beneficial effect on the production of ammonia from cyanamide, dried blood, urea and hongay leaf; the corresponding observations in the cases of farmyard manure, mahua, lantana and straw were not significantly different from those of the controls. The effect of lime was the reverse of that of sulphur though only to a moderate extent. The influence of sulphur on nitrification was not very marked; lime appeared to have a slightly beneficial effect.

The precise manner in which sulphur or lime functions under swamp soil conditions is still obscure. It was found, especially in presence of the different organic substances, that a large part of the added sulphur was reduced to hydrogen sulphide which combined with the iron and other metals present in solution to form the corresponding sulphides. The effect of lime would be modified by the organic acids and carbon dioxide formed during the fermentation.

Effect of increasing quantities of organic matter on the economy of carbon and nitrogen. To 100 gm. lots of soil, dried blood corresponding to 10, 20 and 40 mg. of nitrogen respectively were added, and the attendant changes in total nitrogen, mineralised nitrogen and total carbon followed. It may be seen from Fig. 4 that there is increasing production of ammonia in all the cases, the maximum, which corresponds to about 30 per cent.

of the added nitrogen, being attained between the 29th and 57th days. The loss of nitrogen increases with the quantity of dried blood: thus,

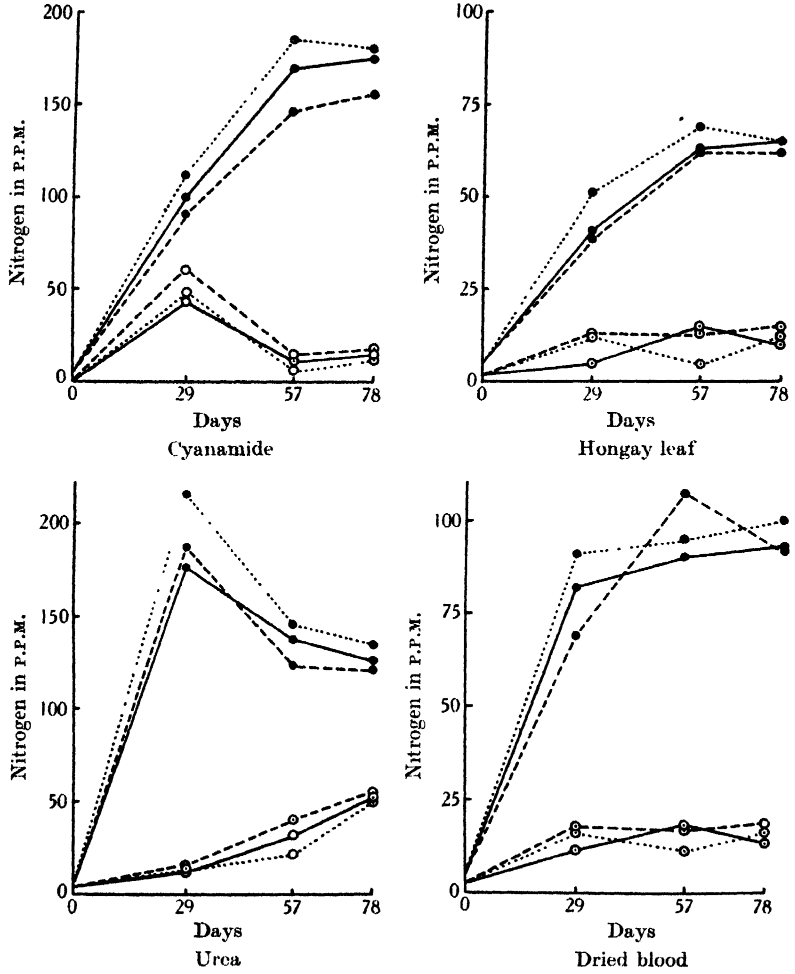
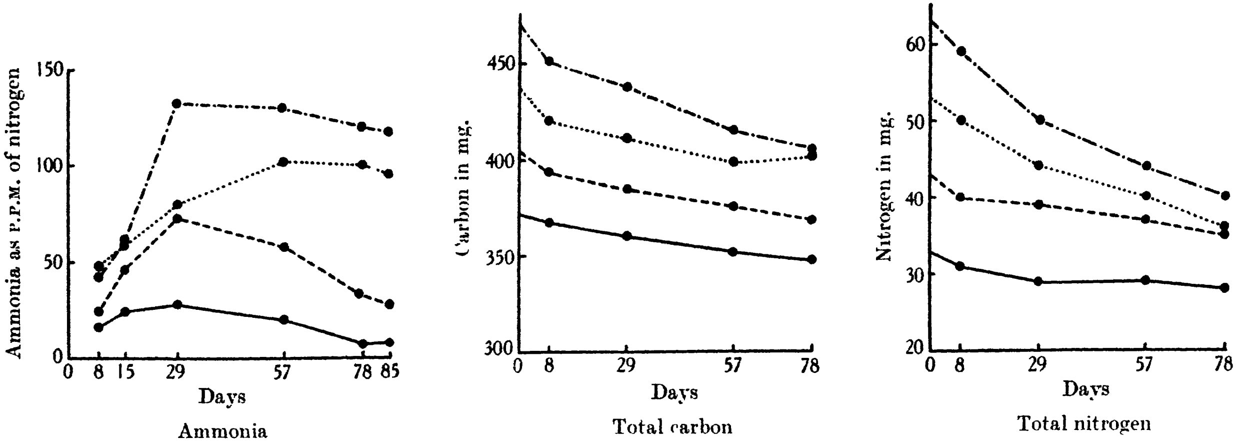


Fig. 3. Influence of sulphur or lime on the mineralisation of nitrogen.

●----● Ammonia in presence of sulphur ○----○ Nitrate in presence of sulphur
●— —● ,, ,, lime ○— —○ ,, ,, lime
●——● ,, control ○——○ ,, control

whereas only 5 mg. of nitrogen were lost when 10 mg. were added, nearly 23 mg. were lost when 40 mg. were added, so that increasing quantities do not confer any added benefit. The changes in C/N ratio (Table IV) show that at all proportions there is a tendency to



Ammonia

Total carbon

Total nitrogen

Fig. 4. Effect of addition of increasing quantities of dried blood.

●——● Soil (100 g.) + 80 mg. of dried blood
●– – –● ,, +160 mg. ,,
●·····● Soil (100 g.) + 240 mg. of dried blood
●–·–·● ,, +320 mg. ,,

equilibrium in the region of 10–11 to 1 as in the cases of other substances Since the attainment of the above equilibrium would necessarily involve considerable wastage, it would be uneconomical to add substances with narrow C/N ratios, except in small lots at a time or in presence of immobilisers which would retain the surplus nitrogen in combined forms

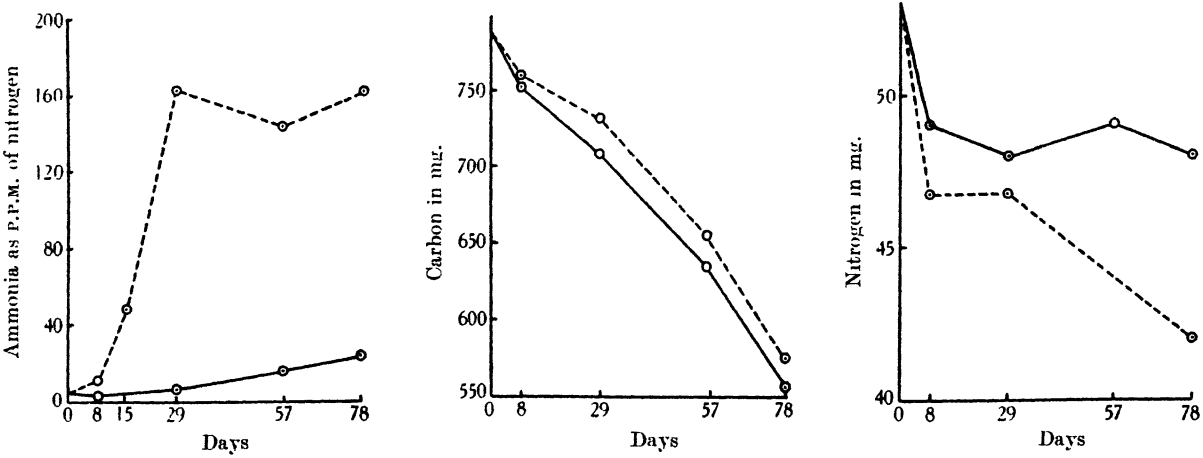
Table IV. *Carbon-nitrogen ratios in presence of increasing quantities of dried blood.*

Dried blood (in mg.) added to 100 gm. of soil	Days				
	0	8	29	57	78
80	11·3	11·9	12·4	12 1	12·4
160	9·4	9 9	9·9	10·2	10·5
240	8·2	8·4	9·3	10·0	11 2
320	7·5	7·6	8·8	9·5	10 2

Effect of addition of mixtures with the same C/N *ratios.* With a view to determining whether the extent of mineralisation and the loss of nitrogen can be adequately controlled by adjusting the ratio of carbon to nitrogen, some experiments were carried out with mixtures of (*a*) lantana (C/N = 22·9) and dried blood (C/N = 3·3), and (*b*) urea (C/N = 0·4) and glucose (C = 40 per cent.). The mixtures were adjusted to the ratio 15 to 1 and quantities corresponding to 30 mg. of nitrogen were added to 100 gm. lots of soil.

A comparison of the results (Fig. 5) with those obtained in the case of single substances (Fig. 1) shows that the production of ammonia from urea was not appreciably checked by glucose, while that from dried blood was greatly retarded by lantana. The quantities of nitrites and nitrates present at different stages were negligible, so that in both the sets nitrification was effectively checked. This effect is markedly seen in the case of urea, which when present by itself nitrified fairly rapidly after the first few weeks (Fig. 2). The presence of glucose had obviously checked biological oxidation beyond the ammonia stage. A further effect of adjusting the C/N ratio is that the loss of carbon from lantana is retarded by dried blood and that from glucose by urea. In a like manner, the loss of nitrogen from urea and dried blood is retarded. In both the sets there is a tendency to reach equilibrium at a lower C/N ratio than at the outset. It is probable that a ratio of about 11 to 1 would be finally attained as in the previous cases.

The nature of factors determining the behaviour of mixtures of organic substances with different C/N ratios and their bearing on the growth of the rice plant are under investigation.



Ammonia

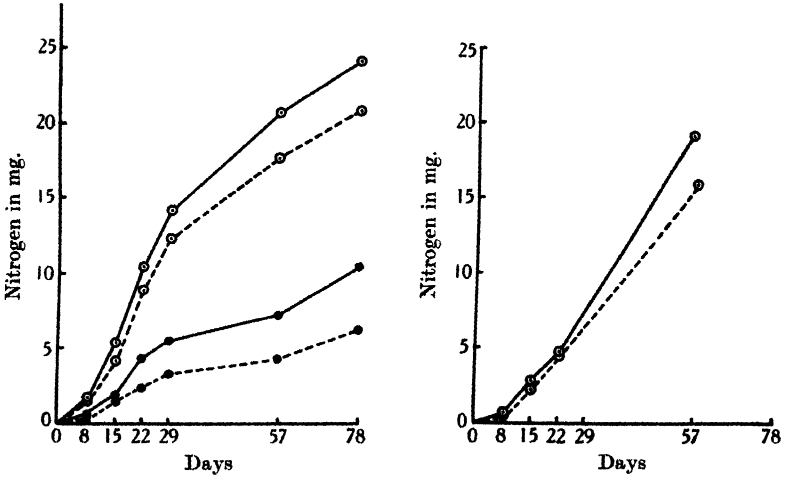
Total carbon

Total nitrogen

Fig. 5. Effect of addition of mixtures of the same C-N ratio.

⊙—⊙ Lantana-dried blood mixture ⊙- - -⊙ Urea-glucose mixture

The form in which nitrogen is lost from the soil system. In view of the previous observation that the loss of nitrogen was greatest in the case of substances which underwent the most rapid ammonification, it appeared probable that a part of the loss might have been due to volatilisation of ammonia. Some experiments were therefore carried out, adding urea or dried blood containing 50 mg. of nitrogen to 100 gm. lots of soil in 250 c.c. conical flasks. After addition of water, each flask was fitted with a rubber stopper which was connected with a gas wash bottle containing standard acid. At stated intervals, a gentle stream of ammonia-free air was drawn through the flasks just above the level of the soil suspension, and the ammonia present in the space, together with that which had been previously absorbed in the wash bottles, was determined by back-titration of unused acid against standard alkali. Changes in total nitrogen were also followed at corresponding intervals. The results were compared with those from similar experiments carried out with soil treated with dried blood but maintained at 60 per cent. saturation.



Dried blood under swamp and arable soil conditions

Urea under swamp soil conditions

Fig. 6. Loss of total nitrogen and volatilisation of ammonia.

○—◉ Total nitrogen lost under swamp soil conditions
○- -◉ Ammonia nitrogen volatilised under swamp soil conditions
●—● Total nitrogen lost under dry soil conditions
●- -● Ammonia nitrogen volatilised under dry soil conditions

It may be noted (Fig. 6) that there is fairly close agreement between the quantities of ammonia volatilised and the total nitrogen lost from the soil. The loss is very much greater from the water-logged specimens than from those maintained at 60 per cent. saturation.

Attention has already been drawn to the fact that in the water-logged soil ammonification proceeds at a very much faster rate than nitrification. This would naturally result in the accumulation of ammonia which would volatilise and thus be lost from the soil system. In the present experiment, the temperature was 30° C., which is very much less than those prevailing in many parts of the tropics. It may be expected therefore that, under field conditions, volatilisation of ammonia would proceed at even a faster rate than that recorded in the present experiment. The nitrogen lost in this manner would naturally be quite considerable in the case of substances with narrow C/N ratios which would ammonify rapidly. With such substances added in increasing quantities, the production and subsequent volatilisation of ammonia would become correspondingly large. This would explain the previous observations with dried blood (Fig. 4).

It has already been noted that addition of glucose is fairly effective in checking the volatilisation of ammonia (Fig. 5). This may at first appear obscure, but a critical study of the related changes would show that the decomposition of urea and glucose in the water-logged soil proceeds independently, at any rate in the early stages, so that while the former yields ammonia, the latter produces organic acids (Subrahmanyan (19–21)). This would naturally result in the two products combining to form salts which would be retained in the soil system. The addition of sugar would also appear to have encouraged the growth of micro-organisms which assimilate a part of the mineralised nitrogen and retain it in their body material.

Following the classical work of Gayon and Dupetit (5) on denitrification in sewage and in biological media, a number of workers have suggested the possibility of similar changes occurring under the "anaerobic" conditions of swamp soils. There is not much experimental evidence, however, to support such a theory. The typical swamp soil seldom contains more than traces of nitrates, so that denitrification, as commonly understood, has no scope to play any large part under such conditions. Furthermore, as may be seen from the results of the present study, there is very little loss of nitrogen during the early stages of fermentation when the dissolved oxygen content of the medium is low (Subrahmanyan (19–21)) and the conditions are favourable for anaerobic changes. It is only after the first few weeks, when aerobic conditions are restored and nitrification sets in (Fig. 1), that the loss of nitrogen from the soil system is most pronounced. It would thus be seen that the volatilisation of ammonia proceeds under conditions which are the reverse of those favouring denitrification.

Although the foregoing observations show conclusively that volatilisation of ammonia is an important factor in determining the loss of nitrogen from swamp soils, further work is yet needed to show whether that is the chief form in which nitrogen is lost under all conditions. The nitrogen transformations that occur in the soil both in presence of the growing plant and after the harvest of the crop require systematic enquiry. The observations of Harrison and Aiyer (7–10), that elementary nitrogen is among the gases evolved from the swamp soil, also require an explanation.

The results obtained from the specimens maintained at 60 per cent. saturation show that loss of nitrogen by volatilisation of ammonia proceeds under dry-soil conditions as well. This is supported by the previous observations of Gundu Rao (unpublished data), who noted that even at 25–30° C. ammonia tends to volatilise from the soil, and that in the case of soils treated with ammoniacal fertilisers a part of the nitrogen is generally lost in that form.

SUMMARY.

1. During the fermentation of water-logged soil containing added substances with different carbon-nitrogen ratios, the reaction first turns slightly acid, but soon returns to the original hydrogen-ion concentration (*p*H 7·6).

2. The quantities of ammonia present in the medium increase up to a point, after which there is steady decrease.

3. There is nitrification only in the case of substances with narrow C/N ratios. The production of nitrate generally commences only after about a month, when the vigour of the initial fermentation has subsided and fairly large quantities of ammonia have accumulated in the medium.

4. The extent of mineralisation of nitrogen is determined chiefly by the C/N ratio, though in the cases of substances like mahua and lantana the presence of other constituents may also influence the processes. The quantities of mineralised nitrogen present in the soil system generally tend to decrease after about two months.

5. Carbon is lost from all the added materials, especially those with wide C/N ratios. Substances with narrow C/N ratios lose more nitrogen than the others.

6. There is practically no formation of complex nitrogenous bodies

from substances with narrow C/N ratios, the major part of the added nitrogen being either mineralised or otherwise lost from the soil system. Such substances will therefore have no residual value. On the other hand, materials with wide ratios mineralise very slowly; they are mostly present as complex forms, so that although they possess very little immediate fertilising value, their residual effect in the soil may yet be high.

7. The loss of total nitrogen observed, especially with urea and dried blood, is largely accounted for by volatilisation as ammonia from the soil system. Under the swamp soil conditions ammonification proceeds at a very much faster rate than nitrification so that there is accumulation of ammonia in the medium. The volatilisation of ammonia is favoured by the high temperature prevalent under tropical conditions. A similar, though less heavy, loss was also observed under dry soil conditions, accounting for over 60 per cent. of the total nitrogen lost from the soil system.

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(*Received August 20th*, 1934.)

THE CALCIUM, PHOSPHORUS AND VITAMIN D REQUIREMENTS OF SWINE.

By GEORGE DUNLOP.

Ministry of Agriculture Research Scholar.

(*From the Animal Nutrition Research Institute, School of Agriculture, University of Cambridge.*)

(With One Text-figure.)

INTRODUCTION.

RESEARCH work in the nutrition of domestic animals has of late extended its scope to a study of the recently discovered food factors and "trace" minerals shown to be necessary for normal growth and development in the animal organism. Many investigations have now been conducted with diets which contain the minimum possible amount of a certain element or organic compound, and in most cases it has been possible to demonstrate that on such diets a condition of stunted growth and profound emaciation arises in the animals. It is true that, under apparently similar conditions at other centres, confirmatory evidence has not always been forthcoming. Nevertheless, certain mineral- and vitamin-deficiency diseases experimentally producible in the laboratory rat have been shown beyond doubt to occur under practical conditions of live-stock husbandry. The occurrence of any of these deficiency diseases in farm animals was not convincingly established before the year 1920, and it is necessary here to give a brief review of their discovery.

Following up Mellanby's brilliant researches, Elliott, Crichton and Orr in 1922 showed that swine were liable to develop rickets on low-calcium rations. Hart, Steenbock and Fuller found that rations with a low content of phosphorus produced a similar result, while just previously Theiler and co-workers had shown that the disease, lamsiekte, in cattle, was primarily due to aphosphorosis. In 1923 MacGowan and Crichton demonstrated that an insufficiency of iron in the diet of piglings was prevalent under practical conditions of husbandry and gave rise to anaemia often followed by the death of the animal. Since then evidence has accumulated that the disease is also common in other species of animals living in iron-deficient areas located in various parts of the world.

Over a decade ago lack of iodine in various parts of the middle west of America and in British Columbia was shown, by Marine and Keith respectively, to be the cause of much ill-health both in humans and in live stock. Chlorine deficiencies under winter conditions of management are also known.

The history of the vitamin-deficiency diseases in domestic live stock dates back to a similar period. In 1920 Morrison and co-workers demonstrated the difference in the growth-promoting properties of white and yellow corn, and ascribed the difference to the yellow pigmentation of the coloured variety. It was not till six years later, however, that Rice *et al.* presented conclusive evidence that swine are liable to develop a condition of avitaminosis A. A similar condition has recently been found in cattle and horses by Gmeider in Germany, Hart *et al.* in America and Klemola in Holland. With regard to vitamin D, Zilva, Golding and Drummond in 1921 produced a condition of osteoporosis in swine which could be cured by cod-liver oil therapy. Since then, work at the Rowett Institute and the experiments of Bechdel and others have shown that rickets or osteoporosis can be induced at will in sheep and cattle. Conditions of avitaminosis B in swine and vitamin E deficiency in cattle have been demonstrated more recently by Blissett and Golding at Reading, England, and Vogt-Møller and Bay in Denmark.

Despite the comparatively long time these diseases have been known, when measured in terms of the present-day strides in scientific knowledge, our conception of the amounts of the various nutrients necessary for the health of our species of live stock remains on an unsatisfactory basis. While conditions of mineral deficiency and symptoms of avitaminosis frequently occur, the reverse picture of salt poisoning and hypervitaminosis is not unknown. These abnormalities arise through our ignorance of the optimum concentrations of the food factors which should be present in the diet to produce a normal physiological state in the animal organism. It is the purpose of this paper to provide some indication of the magnitude of the requirements of swine for calcium, phosphorus and vitamin D to give normal bone formation, and at the same time to allow for an adequate rate of growth and development.

EXPERIMENTAL.

The present investigation was commenced in 1931. Experiments have since been conducted on five generations of swine from the time of weaning till they reached bacon weights. In connection with these experiments consideration has been given to various factors, especially to the influence of supplements of vitamin D on the rate of growth and the feed requirement of growth (economy of gain) as well as on the normality of the blood picture. The morphological appearance and chemical composition of the bone at the conclusion of the experiment have also been considered, and in certain of the series the study of the blood picture included Hb determinations and red, white and differential white blood cell counts. These blood counts were made with a view to ascertaining whether the conditions of abnormal bone development and anaemia, frequently met with when animals are deprived of natural radiation and bone-forming materials, are in any way related. A consideration of these factors in the interpretation of the results would appear to show that laborious metabolism balance trials should be unnecessary and would merely serve to upset the normal progress of some of the experimental animals. Consequently such trials were not attempted.

The first experiment was essentially preliminary in nature. It was desirable in the first place to obtain some information on the effect of allowing the animals free access to the bone-forming materials—calcium (Ca) and phosphorus (P)—and certain groups were treated in this manner. The well-known relationship between ultra-violet light and vitamin D, first shown in 1924 by Steenbock and Nelson, must also be considered in experiments of this kind. An attempt was therefore made to ascertain the influence of sunlight as compared with cod-liver oil feeding. Some information was also required as to whether the method of feeding the animals was suited to the requirements of the study. Previous work at other centres gave some indication that the group-feeding method lacked precision and would prove to be unsuitable for accurate work.

EXPERIMENT 1.

In this experiment six groups containing six pigs each were used, all were pure-bred Large Whites from the Cambridge University Farm herd, as were all the animals in our later experiments. In the arrangement of the groups as even a distribution as possible was made with respect to such factors as age, size, sex, type, genetic relationship and general condition. The animals were housed on one side of a Danish piggery and care was taken to exclude all sources of direct sunlight. Straw was used as bedding. The rations were hand fed twice daily, soaked in water and in such amounts as constituted a full feed. Drinking water was supplied *ad lib.*

Experimental treatments.

The arrangement of the various groups from the standpoint of rations and experimental conditions was as under:

Lot 1. Basal ration.
2. Basal ration + ground limestone—minerals *ad lib.*
3. Basal ration—cod-liver oil.
4. Basal ration + ground limestone—minerals *ad lib.*—cod-liver oil.
5. Basal ration—sunlight.
6. Basal ration + ground limestone—minerals *ad lib.*—sunlight.

Cod-liver oil was fed to groups 3 and 4 at the rate of 10 ml. per head daily and was mixed with the ration prior to feeding. Ground limestone was added to the basal ration at the rate of 1·5 lb. to 100 lb. of the basal ration. Groups 5 and 6 were placed in an outside enclosure on suitable days and a record kept of the number of hours of sunshine to which the animals were exposed.

The following foodstuffs constituted the basal ration in this experiment: ground oats 30 parts, ground barley 35 parts, white sharps (wheat offals) 30 parts, meat meal 2·5 parts, linseed-oil meal 1·5 parts, alfalfa meal 1 part. One-half part of common salt (NaCl) was added to every 100 parts of the mixture. On the basis of subsequent analysis of the various foodstuffs the ration was found to have a nutritive ratio of approximately 1 : 5·3. The percentage of Ca was 0·29 and the Ca/P ratio 1/1·95 (calculated as the percentages of Ca, as such, and P, as such, in the ration). When ground limestone was added in the above amounts the Ca level was raised to 0·84 per cent. and the Ca/P ratio became 1/0·64.

For determination of the mineral constituents in the basal diet small representative samples were taken from the batches of the various foodstuffs before mixing. The sample was stored along with samples of previous batches of the foodstuff in air-tight bottles. At the end of the experiment a representative sample was then taken from each bottle and analysed for Ca, P and Mg. The average results obtained for the various foodstuffs in these experiments are given in Table I.

Live-weight data.

The increase in live weight of the individual animals during the experiment is shown in Table II. Group differences are comparatively small for experiments of this nature and bear out the results of our clinical observations—that signs of gross malnutrition did not arise in any of the groups. The only difference which is statistically significant ($t=0·05$) is that between the gains of groups 1 and 4. The inclusion of cod-liver oil in the ration of the latter group raised its energy content by at least 1 per cent., and this no doubt accounts for part of the difference.

Table I. *Composition of foodstuffs.*

The amounts of Ca, P and Mg in the foodstuffs are expressed as percentages of CaO, P_2O_5 and MgO.

Methods: Ca—MacCrudden (permanganate titration).
P—Richards-Godden modification of Pemberton-Neumann method.
Mg—Washburn and Shear.

Foodstuff	CaO %	P_2O_5 %	MgO %
Ground barley	0·067	0·87	0·176
Ground oats	0·147	1·23	0·274
White Sharps	0·071	1·38	0·376
Meat meal (Exp. 1)	12·233	11·34	0·933
Meat meal (Exp. 2)	3·675	3·47	0·362
Linseed cake meal	0·469	1·62	0·790
Alfalfa meal	0·560	1·27	0·310
Soya bean meal	0·347	2·23	0·265
Ground limestone	53·62	—	1·42

Table II. *Live-weight gains of individual animals.*

Initial weight = live weight on October 19. Final weight = live weight on February 23. Experimental period—126 days.

	Initial weight	Final weight	Gain	Initial weight	Final weight	Gain
		Group 1			Group 2	
	45·5	204·0	158·5	46·5	156·0	109·5
	52·5	212·0	159·5	45·5	217·0	171·5
	46·5	172·5	126·5	52·0	197·5	145·5
	45·0	191·5	146·5	47·0	185·5	138·5
	44·5	158·0	113·5	41·0	208·0	167·0
	52·5	185·0	132·5	53·0	203·0	150·0
Av.	47·8	187·2	139·4	47·6	194·5	146·9
		Group 3			Group 4	
	57·0	267·0	210·0	49·5	219·5	170·0
	44·5	208·0	163·5	48·5	220·5	172·0
	46·5	201·0	154·5	58·5	240·5	182·5
	45·5	183·5	138·5	41·0	169·0	128·0
	48·0	131·0	83·0	45·5	177·0	131·5
	47·5	210·5	163·5	46·0	198·5	152·5
Av.	48·1	200·3	152·2	48·2	204·2	156·0
		Group 5			Group 6	
	48·5	235·5	187·0	46·5	219·0	172·5
	42·5	189·0	146·5	47·5	228·0	180·5
	47·0	198·5	151·5	44·5	162·5	118·5
	39·0	146·0	107·0	47·5	196·0	148·5
	46·5	191·0	144·5	49·0	207·0	158·0
	62·0	250·0	188·0	52·5	166·5	114·0
Av.	47·6	201·5	153·9	47·8	196·5	148·7

The group gains on the rations used were normal for winter conditions, being 1·10, 1·15, 1·21, 1·23, 1·22 and 1·18 lb. per day respectively. The chief point of interest about the live-weight data, however, is the great individual variation. This point will be returned to later.

Blood analyses.

Samples of blood were drawn from each animal at monthly intervals. For this purpose the animal was confined to a special crate. The tail was thoroughly cleaned with lukewarm water and rubbed with cotton-wool slightly damped with methylated spirit. 1–2 mm. of tissue was then snipped off the end of each tail and a steady stream of blood was obtained. The sample for estimation of Ca was run into a special centrifuge tube and tightly corked. For the blood inorganic P determination the sample was collected in a small bottle containing finely ground oxalate in the proportion of 0·1 ml. of 20 per cent. potassium oxalate to 5 ml. of blood. The bottle was thoroughly shaken to avoid clotting. One ml. of blood was immediately measured out, deproteinised with 10 per cent. trichloracetic acid and the filtrate retained for analysis. The oxalated blood also served for the determination of Hb and blood-cell counts.

The methods used in the analyses were: serum Ca—Kramer and Tisdall; blood inorganic P—Fiske and Subarrow; serum phosphatase—Bodansky; Hb—Barcroft. For red blood-cell counts the blood was diluted 1 : 200 in Ostwald pipettes with Ringer's solution. For white blood cells the blood was diluted 1 : 10 in similar pipettes with a 0·2 per cent. acetic acid solution to which Leishmann's solution was added for staining purposes. A Bürker haemocytometer was used for counting purposes.

Analyses of the preliminary samples of blood showed that all groups had normal values for the various constituents under investigation (Table III). Thereafter the blood picture continued normal in all groups except group 1. The animals in this group showed a slight decrease in serum calcium which was correlated with a reciprocal rise in blood P as the experiment progressed. The actual values, however, are not comparable with those found in cases of acute hypocalcaemia or hyperphosphataemia, but nevertheless the deviation was sufficient to show that the true Ca, P and vitamin D requirements of the animals had not been satisfied.

The numerical counts of the erythrocytes were found to be correlated with the amount of Hb present in the blood. But since these counts and also enumeration of white blood cells were found to be of little diagnostic value in the present study they need not concern us now and will be reported elsewhere. Cell counts during the development of rickets have been shown by other workers to remain fairly constant (9, 16). Suffice it to say that in later experiments abnormally high and abnormally low counts for both types of blood cells were found at times in advanced stages of rickets.

Table III. *Blood analyses, Exp.* 1.

Serum Ca—mg. per 100 ml. serum. Blood inorganic P—mg. per 100 ml. blood. Hb—gm. per 100 ml. blood.

Group		Oct. (prelim.)	Nov.	Dec.	Jan.	Feb.
1	Serum Ca	11·25	10·28	9·96	9·91	9·73
	Variation	10·8-12·0	9·8–11·1	9·5–10·7	9·5–10·2	9·2–10·0
	Blood P	6·55	5·73	5·80	6·46	5·8
	Variation	5·7–7·7	5·1-6·2	5·5–6·3	6·1-6·7	5·2–6·2
	Hb	10·4	11·7	11·7	12·3	12·9
2	Serum Ca	11·10	10·68	10·56	10·47	10·53
	Variation	10·5-11·6	10·3–11·0	10·3–10·9	10·4-10·7	10·4–10·7
	Blood P	6·43	5·44	5·34	5·51	5·07
	Variation	5·5–7·5	4·9-6·0	4·8–5·8	4·9–5·8	4·6-5·3
	Hb	10·7	12·2	12·1	12·4	13·0
3	Serum Ca	11·60	10·66	10·22	10·35	10·34
	Variation	10·9-12·5	10·3–11·1	9·9–10·5	9·9-10·7	10·1–10·6
	Blood P	6·09	5·71	5·80	6·11	5·87
	Variation	4·7–8·0	5·5–6·3	5·1–6·6	5·7–6·7	5·6–6·5
	Hb	10·2	11·7	11·9	12·10	12·8
4	Serum Ca	11·17	11·04	10·60	10·66	10·77
	Variation	10·3 11·9	10·5–11·7	10·2–10·9	10·4-11·0	10·5 11·2
	Blood P	6·67	5·26	5·07	5·59	4·97
	Variation	5·6–7·7	4·6–5·6	4·2–5·8	5·3–6·1	4·4–5·3
	Hb	10·3	11·8	12·2	12·4	13·1
5	Serum Ca	11·98	10·60	10·47	10·35	10·11
	Variation	10·8–13·4	10·0–11·3	9·7–11·4	9·8–10·8	9·8–10·4
	Blood P	6·32	5·78	6·09	6·03	5·59
	Variation	5·4–6·8	5·4–7·3	5·2–6·8	5·5–6·9	5·1–6·1
	Hb	10·6	11·4	11·9	12·3	12·7
6	Serum Ca	12·01	10·85	10·60	10·75	10·77
	Variation	11·4–12·5	10·3–11·0	9·8–10·9	10·6–11·0	10·6–10·9
	Blood P	6·62	5·38	5·39	5·52	5·22
	Variation	4·6–8·3	4·7-6·0	4·7–6·5	5·1–6·0	4·7–5·6
	Hb	10·6	11·5	12·0	12·2	12·9

Discussion.

The blood picture of the animals shows that addition of minerals, or cod-liver oil, or exposure to sunlight was sufficient to correct the deficiency of the basal ration (cf. group 1 with groups 2, 3 and 5). It was not possible to demonstrate the relative merits of these treatments as this deficiency was only slight and proved insufficient to give rise to symptoms of malnutrition in the animals. A limited amount of winter sunshine, however, appears to be able to replace cod-liver oil as a source of vitamin D when the need for its administration is not great (cf. groups 3 and 5). Free access to minerals (limestone, steamed bone flour, ferric oxide and charcoal) gave a normal blood picture with no deleterious effects in the animals (group 2). The effect of providing both minerals and sources of vitamin D was no more marked than when these adjuvants were supplied separately (groups 3 and 4 and groups 5 and 6).

The live-weight data prove conclusively that the method of feeding the animals is unsuitable for an accurate study of their mineral and vitamin requirements. The variability in the growth rates of animals on the same treatment was disconcertingly great. To obtain significant results between treatments which differ only slightly in the effects they produce it is essential to reduce variation to a minimum. It is clear that the differences in the growth rate must be due to the varying feed intake of the animals. Range in initial weight at the beginning of the experiment may be a contributory cause although lesser in its effects. In a comparison of treatments, which differ little in the effects they produce, it becomes necessary therefore to correct for varying initial weight and to control the food intake of the animals. To accomplish this a new technique was evolved which has formed the subject of a previous communication (6), and the investigation was continued on these lines.

EXPERIMENT 2. SUMMER, 1932.

Although no definite conclusions could be drawn from the live-weight data in the preliminary experiment, consideration of the blood picture showed that the basal ration just failed to meet the requirements of the animals for Ca. To find the effect of measured doses of vitamin D a lower level of Ca had to be employed. This was accomplished by including in the ration a brand of meat meal containing a lower percentage of ash.

Thirty animals from seven litters were used in the experiment. No animal had reached 40 lb. when the experimental rations were first fed. Five animals were allotted to each of the six treatments. The general arrangement of the experiment is given in Table IV.

The method adopted in feeding the animals and the plane of nutrition imposed are given elsewhere (6).

Routine clinical examination of the blood was carried out as before. At slaughter the tibia of the left hind leg of each animal was obtained for ash determinations on the dry-fat free bone.

Table IV. *Showing the treatments in Exp.* 2.

Group	Parts $CaCO_3$ added to 100 parts ration	Daily supplements: Olive oil ml.	Daily supplements: Cod-liver oil* ml.	Ca %	P %	Ca/P
1	—	20	0	0·15	0·53	1/3·7
2	—	15	5	0·15	0·53	1/3·7
3	—	—	20	0·15	0·53	1/3·7
4	½	20	0	0·34	0·53	1/1·6
5	½	15	5	0·34	0·53	1/1·6
6	½	—	20	0·34	0·53	1/1·6

* 300 I.U. per gm.

Live-weight data.

Although the experimental rations were fed shortly after weaning, the animals were not rationed strictly in accordance with their live weight till some time afterwards. When this occurred, one of the animals weighed 64 lb., which has therefore been taken as the datum in considering the results for growth in weight. These are given in Table V.

Table V. *Live-weight records in Exp.* 2.

Results expressed as actual live weights 11 *weeks after reaching* 64 *lb.*

Litter No.	Group 1	2	3	4	5	6
1	—	—	152	149	155	148
2	148*	158	161	160	161	159
	—	158	—	161	—	—
3	134*	—	160	—	—	—
4	134*	159	—	—	—	—
5	—	—	155	—	153	153
6	155	158	133*	154	157	165
7	136*	155	—	158	156	157
Normal animals	1	5	4	5	5	5
Average gain (av. weight—64)	91·0	93·6	94·7	92·4	92·4	92·4

General mean 92·7. Standard error 1·87.

* Indicates that animal adopted lower plane of nutrition.

All the animals in group 1 developed marked symptoms of rickets. After having adopted the plane of nutrition imposed for some weeks they began to consume less food than the amount offered—as determined by their live weight. The weekly gains in weight diminished till no further increase in weight took place. The live weights at which growth ceased were 196, 159, 140, 171 and 142 lb. respectively. Only one animal (that from litter No. 6) consumed its total allowance of food for 11 weeks after reaching 64 lb. During this period its gain in weight was similar to the gains in the other groups. In considering the economy of gains, this animal provides the only observation in group 1, but it is sufficient to show that no significant differences exist between the treatments as far as utilisation of the food is concerned. This result may be expected, since the energy content of the rations was for all purposes the same, the only difference being that the ration fed to groups 4, 5 and 6 contained 0·5 per cent. less gross energy as a result of the amount of limestone added.

As noted above, appetite and growth rate declined progressively in all animals in group 1. On the other hand, the individuals in the remaining groups (apart from one in group 3) consumed the whole of their food allowance and grew normally throughout the experiment.

Blood analyses.

The results obtained in the study of the blood picture are given in Table VI. Almost from the outset there was a marked drop in the serum Ca of group 1 animals. When the values fell below 8 mg. per 100 ml., symptoms of rickets became very noticeable. An animal which had lost weight during the previous week showed the lowest value of 5·90 mg. Cod-liver oil therapy in conjunction with the higher mineral diet restored serum Ca to normal levels. The more gradual fall in the serum Ca values of groups 2 and 3 showed that even when 6000 international units (I.U.)

Table VI. *Values expressed as in Table III.*

Group		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
1	Serum Ca	10·66	10·20	9·56	7·39	8·04	7·23	6·15	—
	Variation	10·0–11·9	10·0–10·4	9·1–10·0	5·9–8·8	6·9–9·2	6·5–9·4	—	—
	Blood P	6·51	7·46	7·84	8·57	8·02	7·01	6 93	—
	Variation	6·0–7·2	6·4–9·0	6·9–9·1	7·9–9·4	7·5–8·4	5·4–8·9	—	—
2	Serum Ca	10·57	10·03	9·98	9·85	9·66	9·34	9·16	8·29
	Variation	9·5–11·4	10·0–10·1	9·7–10·1	9·6–10·1	9·5–9·9	8·3–9 8	8·7–9·7	7·5–9·5
	Blood P	6·78	7·16	7·14	7·66	7·58	7·38	8·27	8 85
	Variation	6·4–6·9	6·6–8·0	6·2–7·6	7·3–8·0	7·1–8·0	6·9–8·3	8·2–8·5	8·8–9·0
3	Serum Ca	10·21	10·15	10·24	9·85	9·91	9·70	9·25	9·00
	Variation	9·4–11·6	9·6–10·7	9·4–10·4	9·5–10·2	9·5–10·3	9·2–10·0	9·2–9·4	8·8–9·2
	Blood P	6·62	7·49	7·37	7·58	7·49	7·30	7·09	7·36
	Variation	6·0–7·2	6·4–9·1	6·8–8·0	7·0–8·1	7·1–7·7	6·6–7·7	6·8–7·4	7·0–7·7
4	Serum Ca	10·54	10·23	10·27	10·05	10·35	10·19	10·17	10·40
	Variation	10·0–11·2	10·0–10·4	10·1–10·4	9·8–10·2	10·1–10·5	9·6–10·6	9·9–10·5	10·3–10·5
	Blood P	7·20	7·43	7·21	7·14	7·19	6·68	6·74	6 85
	Variation	6·9–7·6	7·0–8·3	6·4–8·0	6·6–7·9	6·8–8·0	6·1–7·3	6·7–6·8	6·3–7·4
5	Serum Ca	10·13	10·22	10·48	10·44	10·20	10·50	10·22	10·79
	Variation	9·2–11·0	10·0–10·5	10·0–10·9	10·3–10·6	10·0–10·4	10·3–10·6	10·1–10·4	10·6–11·1
	Blood P	6·21	6·75	6·71	6·22	6·71	6·53	5·97	6·83
	Variation	5·9–6·5	5·9–7·2	3·9–7·6	4·1–7·1	6·0–7·3	5·8–7·3	5·7–6·3	6·5–7·2
6	Serum Ca	9·75	10·29	10·56	10·56	10·66	10·61	10·16	10·41
	Variation	8·8–10·4	10·0–10·5	10·2–10·9	10·2–10·9	10·3–10·9	9·5–11·6	9·7–10·6	10·4
	Blood P	6·32	7·34	7·30	7·12	7·29	6·55	6·34	6·36
	Variation	5·6–7·4	7·0–8·0	6·3–7·9	6·5–7·4	7·0–7·6	4·7–7·8	6·1–6·6	5·9–6·7

of vitamin D from cod-liver oil were supplied daily over and above the amount in the basal ration, a normal blood picture could not be obtained. Serum Ca of the animals in groups 4, 5 and 6 showed little change from normal values throughout the experiment, and the original levels were maintained.

The blood inorganic P was at a lower level throughout in the groups receiving the limestone supplement. Group 1 showed a picture of true hyperphosphataemia which was only corrected by the mineral and vitamin therapy. The low blood phosphate of group 5 was partly accounted for by one animal which showed a continual state of hypophosphataemia. In general, it may be said that the concentration of blood inorganic phosphate tended to vary inversely as the serum Ca.

Bone analyses.

The ash content of the dry fat-free tibias of the animals is given in Table VII. The ash content of the bones of group 1 animals is significantly lower than that of the other groups, while the values obtained for groups 2 and 3 are significantly less than those for groups 4, 5 or 6. No appreciable differences exist, however, between these latter groups. The results also show that, although the Ca and P of the blood were restored to normal levels soon after changing the treatment of group 1 animals, the ash content of their bones was still low at slaughter.

Table VII. *Exp. 2. Percentage of ash in the dry fat-free tibia obtained from the left hind limb of each animal at slaughter.*

	Group					
	1	2	3	4	5	6
	50·5*	55·2	53·2	55·1	56·8	55·0
	53·9*	53·2	54·8	55·9	55·1	56·2
	51·8	54·0	54·3	55·2	55·3	55·2
	54·7*	54·8	53·2	56·2	56·7	57·2
	55·2*	55·2	54·0	56·4	57·2	55·8
Av.	51·8	54·5	54·0	55·8	56·2	56·0

* After therapeutic treatment.

For the most part the results obtained for analyses of the bone are in accordance with the data for routine examination of the blood.

EXPERIMENT 3. WINTER, 1932–3.

In this and all of the other experiments described later the basal ration was as follows: ground barley 35 parts, ground oats 20 parts, wheat middlings 30 parts, extracted soya-bean meal 15 parts. To every 100 parts of the mixture $\frac{1}{2}$ part of common salt (NaCl) and $\frac{1}{16}$ part of ferric oxide were added.

This ration was later shown to be deficient in vitamin A for normal growth and health in swine, and the findings in this respect have formed the subject of a previous communication (7). This disturbing factor may be corrected by either of two methods: (1) by incorporating vitamin A-rich supplements, such as carrots, halibut-liver oil or green food in the experimental ration, or (2) by ensuring that the animals have a sufficient reserve of vitamin A, before the experiment commences, to allow normal growth till slaughter. Since the latter method is normally adopted in practice and is also the more suitable from an experimental standpoint, all animals received vitamin A-rich supplements early in life and the ration alone was fed during the experiment.

The installation of concrete feeding troughs in place of somewhat shallow metal receptacles was the only other change in the experimental equipment and technique of the previous series.

To the basal ration as given above 0·25 per cent. of limestone was added. This raised the Ca content of the diet to 0·186 per cent. and gave a Ca/P ratio of 1/3·00. Fifteen animals were fed this diet, but later when it became evident that the ration was grossly deficient in essential nutrients for normal Ca and P metabolism five animals received in addition 900 I.U. vitamin D (ostelin) daily, while the other five received twice this dose. The remaining five animals served as controls.

A second lot of fifteen piglings was being used simultaneously in another experiment, but the nature and object of this experiment did not preclude a continuation of the present study. These animals were divided into three groups, primarily on a litter basis, with five animals in each group. The basal ration was fed with the addition of $\frac{1}{2}$ per cent. $CaCO_3$. One group received a supplement of 25 gm. of meat meal per day, a second lot 40 gm. of soya-bean meal and the third lot 100 gm. of raw liver. The respective Ca levels and Ca/P ratios of these three groups were 0·32, 0·28 and 0·29 per cent. and 1/1·8, 1/2·0 and 1/2·0.

Live-weight data.

The results for live-weight increase in these groups are given in Table VIII. The reaction of the animals to the diet containing the lower amount of Ca shows conclusively that it is unsuitable for normal growth in swine. The effect of the addition of vitamin supplements is more apparent in the consideration of the blood picture. It may be mentioned here, however, that the food intake of the animals receiving the supplement improved appreciably towards the end of the experiment, although the normal plane of nutrition was only adopted spasmodically. The groups with the higher amount of Ca in their diet showed normal growth. The greater live weights attained by group 5 animals are probably significant when the effect of litter variation is corrected for. This is apparent from Table VIII (groups 4, 5 and 6), where the first two rows of live-weight data refer to animals from one litter while the other two litters are represented by the data in the next two rows and the last row respectively. The better showing of the group 5 animals is correlated with the larger amount of energy they received in the daily supplement to their diet. The growth rate is somewhat less when compared with the results in the previous experiment in which a ration of similar energy value was used. The utilisation of the food for live-weight increase has obviously been affected by the lower temperature during the winter months.

Table VIII. *Live-weight records in Exp.* 3.

Results expressed as live weights 17 *weeks after reaching* 40 *lb.*

Group	1	2	3	4	5	6
% $CaCO_3$ added to basal ration ...	¼	¼	¼	½	½	½
Supplements ...	—	900 I.U. vit. D	1800 I.U. vit. D	Meat meal	Soya-bean meal	Raw liver
	139*	133*	168	166	172	170
	144*	100*	—	170	176	163
	173	131*	106*	163	171	162
	171	109*	175	173	173	174
	133*	174	114*	159	165	163
Normal animals	2	1	2	5	5	5
Av. gain (av. weight—40)	132	134	131·5	126·2	131·4	126·4

* Indicates that animal adopted lower plane of nutrition.

Blood analyses.

The results obtained in the analyses of the blood are given in Table IX. The vitamin supplements to the diets of groups 2 and 3 were not introduced till some time after the first samples of blood had been analysed. The effect of the supplements in correcting the lowered Ca in the blood of the animals is quite marked. While the serum Ca of the group 1 animals, receiving no supplement, continued to fall, groups 2 and 3 showed increased values towards normal. Nevertheless although the supplements supplied approximately the same number of units of vitamin D as the supplements in the previous experiment their therapeutic effect appears to have been less. The lower efficiency of vitamin D concentrates as anti-rachitic agents when compared with cod-liver oil has been shown previously by other workers (2, 10).

Table IX. *Blood analyses, Exp.* 3. *Values expressed as in Table III.*

Group		Dec.	Jan.-Feb.	Mar.	Apr.
1	Serum Ca	10·10	9·45	9·52	8·78
	Variation	9·8–10·7	8·5–10·3	8·7–9·8	7·8–10·4
	Blood P	7·0	7·22	7·1	6·5
	Variation	6·4–7·1	6·8–7·8	6·8–7·7	5·9–7·3
	Hb	11·3	12·6	12·1	12·3
2	Serum Ca	9·21	8·91	9·47	9·79
	Variation	5·5–10·7	8·2–9·5	8·6–10·6	9·0–10·3
	Blood P	6·8	6·9	6·5	6·0
	Variation	5·9–7·3	6·4–7·7	6·2–6·9	5·3–7·3
	Hb	11·4	12·5	12·5	11·4
3	Serum Ca	9·44	9·28	10·08	10·00
	Variation	8·4–10·1	8·5–9·8	9·4–10·7	8·9–10·6
	Blood P	6·5	6·7	6·9	5·6
	Variation	6·2–6·8	5·5–7·3	6·5–7·2	5·2–6·5
	Hb	11·9	12·7	12·7	11·4
4	Serum Ca	—	10·41	—	10·01
	Variation	—	10·1–10·8	—	7·7–10·9
	Blood P	—	6·84	—	5·90
	Variation	—	6·4–7·4	—	4·0–6·7
	Hb	—	12·6	—	—
5	Serum Ca	—	10·18	—	9·70
	Variation	—	10·0–10·5	—	8·8–11·1
	Blood P	—	6·84	—	6·40
	Variation	—	6·4–7·1	—	5·6–7·3
	Hb	—	12·5	—	—
6	Serum Ca	—	10·56	—	10·04
	Variation	—	10·3–11·2	—	8·3–10·9
	Blood P	—	7·44	—	6·70
	Variation	—	6·9–7·9	—	6·2–7·6
	Hb	—	14·2	—	—

The blood picture of the remaining groups was normal after the rations had been fed for 10 weeks. At slaughter, however, one animal in group 4 and another in group 6 had low serum Ca values, and while the growth rate and food consumption of all group 5 animals was normal to the last, four showed subnormal serum Ca when slaughtered. The remaining animal in this group had the high value of 11·1 mg. per 100 ml. serum.

The Hb determinations showed that anaemia was not a complicating factor in the experiment.

Bone analyses.

The percentage of ash in the dry and fat-free tibia obtained from the left hind limb of each animal after slaughter showed some interesting results (Table X). The serum Ca values at slaughter are included in the table along with the percentage of ash in the bone of the individual animals in groups 4, 5 and 6. A markedly positive correlation is seen to exist between these characteristics. In the other groups the correlation was not noticeable, probably owing to the variable growth rate of the animals.

Table X. *Bone analyses, Exp.* 3. *Percentage of ash in the dry fat-free tibia, obtained from the left hind limb of each animal at slaughter. Serum Ca determinations at slaughter included for groups* 4, 5 *and* 6.

	Group								
	1	2	3	4		5		6	
	Ash %	Ash %	Ash %	Serum Ca	Ash %	Serum Ca	Ash %	Serum Ca	Ash %
	51·6	54·1	54·0	10·6	55·4	9·7	53·7	10·7	55·3
	55·0	53·8	53·6	10·1	54·4	11·1	56·0	8·3	53·6
	55·4	55·4	55·0	10·8	55·6	9·1	54·6	10·0	55·2
	53·2	54·0	54·0	10·8	56·0	8·8	54·9	10·9	56·0
	52·9	56·0	—	7·7	52·7	9·9	54·6	10·4	55·7
Av.	53·6	54·6	54·2	—	54·8	—	54·7	—	55·1

The groups receiving an additional ½ per cent. of limestone show lower values for bone ash than similar groups in the previous experiment. The total amount of Ca in the diet of the latter animals, however, was slightly higher. Considered in conjunction with the blood picture, the bone analyses show that the diets of groups 4, 5 and 6 are inadequate for normal Ca and P metabolism. With regard to the other groups the results of the blood and bone analyses confirm the conclusion which might have been arrived at from the live-weight data alone, namely, that a ration containing 0·18 per cent. Ca is grossly deficient for the nutrition of swine.

EXPERIMENT 4.

In the previous two experiments the investigation had been confined to a study of minimal and subminimal amounts of Ca in the dietary, the P content being apparently adequate. In the present experiment it was decided to determine the effects of higher levels of Ca, the P being kept at the same level as before. For this purpose 2 and 4 per cent. ground limestone was added to the basal ration (p. 32), which gave two diets, with Ca levels of 0·86 and 1·622 and Ca/P ratios of 1/0·65 and 1/0·34 respectively.

Five animals on the low Ca level received in addition 2100 I.U. of vitamin D, a second group of five 900 I.U., while another five received no addition. Five animals on the high Ca level received 4500 I.U. It was also considered advisable to include a control group which was fed a ration of 65 parts barley meal, 25 parts meatings, and 10 parts fish meal.

The experimental treatments are summarised in Table XI.

Table XI. *Experimental treatments, Exp.* 4.

Group	No. of animals	% $CaCO_3$ added	I.U. vitamin D added	Ca (%)	Ca/P
1	5	2	0	0·86	1/0·65
2	5	2	900	0·86	1/0·65
3	5	2	2100	0·86	1/0·65
4	5	4	0	1·62	1/0·34
5	5	4	4500	1·62	1/0·34
6	5	Fish meal	Control	0·79	1/1·20

Unfortunately nine of the animals used in this experiment had not received a diet sufficiently rich in vitamin A before coming on experiment. During the feeding of the experimental rations they developed symptoms of vitamin A deficiency. Consequently it has been necessary to delete their records and the results given here refer only to animals in which the deficiency was not a complicating factor (7).

Live-weight data and utilisation of food.

The live weights of the animals 15 weeks after they reached 50 lb. are presented in Table XII. It will be seen that the gains made by groups 4 and 5 are significantly less than the gains in groups 1, 2 and 3. It seems probable, however, that this is wholly due to the lower energy content of the ration of the former groups, for although the rations were fed in similar amounts to all animals, the greater percentage of limestone included in the high Ca diet depressed its calorific value. In this connection it is interesting to note that Poppov (11) was unable to show any decrease in the digestibility of the food when large amounts of $CaCO_3$ were added to the diet of his animals. That the differences obtained in the present instance are not attributable to the excessive amount of Ca will be shown more clearly in the next experiment.

Table XII. *Live-weight data, Exp.* 4.

Actual live weights of animals 15 *weeks after reaching* 50 *lb.*

Group ...	1	2	3	4	5	6
	180	185	186	173	175	178
	184	189	182	165	174	188
	187	179	184	163	170	191
	182	—	—	168	—	176
Normal animals (%)	100	100	100	100	100	100
Av. gain	133·25	134·3	134·0	117·25	123	133·25

The experiment was commenced in November 1933. The technique of feeding the animals and the methods of routine clinical blood sampling and analysis were as before.

Live-weight data and utilisation of food.

A noticeable feature of these data (Table XV) is the slower growth made by the two groups on the high Ca levels compared with the gains obtained on the same rations the previous summer. This difference is again ascribable to the larger amount of energy required to maintain body temperature under winter conditions.

Table XV. *Live-weight data, Exp.* 5.

Live weights of animals 13 *weeks after reaching* 80 *lb.*

Level of Ca...	0·090		0·284		0·473		0·856		1·62	
Ca/P ratio ...	1/6·3		1/2·0		1/1·18		1/0·65		1/0·34	
	[173]	—	187	3·1*	194	2·4*	176	3·0*	196	1·9*
	[106]	—	188	*C*	193	*A*	186	*A*	175	2·6
	[102]	—	194	*C*	187	*C*	180	*C*	[96]	—
	[87]	—	(*168*)	2·9	185	*C*	192	*A*	[66]	—
	[96]	—	(*176*)	3·1	181	3·0	190	*A*	173	3·0
	202	2·0*	—		183	3·0	174	2·9	—	
	—		—		186	*C*	195	2·0	—	

* Measurement of back fat in centimetres. Letters refer to grader's measurement of back fat at shoulder *A* = under 5 cm., *C* = over 5 cm.

[ ] Stopped growth at this live weight.

( ) Figures in italics indicate that animal assumed a lower plane of nutrition.

A more detailed analysis of the data has been given in another communication ((8), Tables III and IV) which should be referred to. From a study of these tables it becomes clear that the level of Ca has no effect on the utilisation of the food. The observed decrease in live-weight gains as the level of Ca in the diet increases can be accounted for by the decrease in the energy content of the ration through the addition of limestone. It is true that the relationship between decrease in live-weight gain and decrease in the energy content of the ration is not strictly quantitative but the number of observations is too small to expect a more exact picture especially with the arbitrary factor used in correcting the observed live-weight data.

While it has been shown that the utilisation of the food was unaffected by the level of Ca, the diets showed differences in their effects on the growth and general appearance of the animals. On the diet with no added limestone (0·09 per cent. Ca) the animals began to refuse a certain proportion of their daily ration of food. The weekly gains diminished till the live weight became stationary and later declined. One animal was dosed with cod-liver oil when 70 lb. live weight and increased in weight to 173 lb. before its live weight remained stationary. The other animals, however, stopped growth at about 100 lb. The limbs became stiff and deformed and the gait slow and painful. The symptoms were similar to those encountered in rickets.

The group on the high level of Ca presented a somewhat similar picture. The limbs of one animal became very deformed when still consuming the whole of its daily ration. The case again appeared to be one of true rickets. Another after reaching 55 lb. live weight made no gain for 2 months. On the other hand, two animals, long and "rangy" in type, reached bacon weights and had normal appetites throughout the experimental period.

When animals on the low level of Ca began to lose weight, they received the high Ca diet and, conversely, the animals which had all but succumbed to the high Ca diet were switched over to the diet containing the low level of Ca. The apparently anomalous result was obtained, for some time at least, of animals suffering from symptoms of rickets, recovering and making normal growth on another rickets-producing diet. Two animals originally on the low level of Ca and one on the high level recovered and reached bacon weights on this treatment alone, but three others required vitamin D therapy in addition during the later stages. Of these three, the animal which received the high Ca diet showed little improvement in progress on addition of the vitamin supplement.

Animals on the second lowest Ca level (0·28 per cent.) reached bacon weights without supplementary treatment. In three of the animals food consumption became somewhat variable, which suggested that the level of Ca in their diet was insufficient for normal appetite and growth. Only one animal had an absolutely normal appetite during the whole of the experiment.

The appetite, general appearance and growth rate of the remaining two groups were normal throughout.

Blood analyses.

The blood picture showed several interesting features (Table XVI).

The low Ca diet was shown to give rise to abnormal concentrations of the blood constituents. The gradual shift to abnormal serum Ca and blood P values was shown previously with a similar diet (group 1, Exp. 2). In the present instance, however, it is seen that the shift was accompanied by excessive amounts of phosphatase in the blood. The

Jan.-Feb. figures, especially, must be considered as highly significant, since an increase occurred when the animals had ceased to grow in weight—a condition which, ordinarily, has been shown by Bodansky and Jaffe (3) to cause an actual fall in the concentration of the enzyme. On changing the diet of these animals to the high Ca ration the blood inorganic P and serum phosphatase dropped to lower levels accompanied by a mild hypercalcaemia. Addition of vitamin D to the diet at this stage caused the blood constituents to return to normal concentrations (Table XVI (4)), while animals receiving no such supplement continued to show the same abnormal blood picture (Table XVI (1)).

Table XVI. *Blood analyses, Exp. 5. Values expressed as in Table III.*

Ca level and Ca/P ratio		Nov.	Dec.	Jan.-Feb.	Mar.	Apr.
0·090 % Ca	Serum Ca	11·18	9·09	8·04	13·08 (1)	12·29 (1)
1/6·3	Variation	10·9–11·6	8·3–9·9	7·8–8·6	12·1–14·5	12·2–12·4
	Blood P	6·4	6·1	6·3	4·3	5·3
	Variation	5·8–6·9	5·8–6·5	4·7–7·2	4·0–5·0	4·6–6·1
	Phosphatase	11·0	8·6	12·5	9·0	9·4
	Hb	9·8	11·2	11·1	12·7	—
0·28 % Ca	Serum Ca	11·05	11·21	11·26	10·56	11·59
1/2·0	Variation	10·7–11·6	10·7–11·9	10·9–11·6	10·1–10·9	10·7–12·5
	Blood P	5·0	5·8	6·6	6·0	6·5
	Variation	4·0–5·6	4·7–6·9	—	5·7–6·4	5·5–7·5
	Phosphatase	11·5	7·1	6·0	6·1	3·8
	Hb	9·9	10·8	10·0	12·1	—
0·47 % Ca	Serum Ca	11·45	11·13	—	11·06	11·66
1/1·18	Variation	11·0–12·2	10·3–12·0	—	10·4–11·8	10·8–12·4
	Blood P	5·84	5·2	—	6·4	7·2
	Variation	5·5–6·5	4·2–5·7	—	5·7–7·3	5·5–8·2
	Phosphatase	11·6	7·0	—	7·9	5·7
	Hb	9·7	11·2	—	11·8	—
0·86 % Ca	Serum Ca	11·50	12·01	—	12·27	12·38
1/0·64	Variation	11·2–12·0	11·3–13·3	—	11·5–13·6	11·7–13·3
	Blood P	6·5	5·1	—	6·0	6·3
	Variation	5·9–7·4	4·4–5·8	—	5·4–7·0	4·0–7·3
	Phosphatase	13·5	9·1	—	8·0	6·1
	Hb	10·3	11·1	—	11·9	—
1·62 % Ca	Serum Ca	10·95	12·01	13·4	14·5	14·95
1/0·34	Variation	10·8–11·3	11·7–12·4	13·2–13·6	13·3–15·7	14·2–15·7
	Blood P	5·8	4·2	5·3	4·6	5·8
	Variation	5·3–6·3	4·0–4·5	4·9–5·7	4·5–4·7	5·4–6·3
	Phosphatase	11·6	19·6	12·1	9·5	10·4
	Hb	9·9	11·1	12·2	12·9	—
	Serum Ca	10·71 (2)	8·2 (2)	12·37 (3)	11·53 (4)	—
	Blood P	6·6	6·8	4·9	5·8	—
	Phosphatase	8·8	9·3	5·3	5·1	—
	Hb	12·4	—	—	—	—

(1) Changed from treatment 1 to treatment 5. (2) Changed from treatment 5 to treatment 1, data for March and April respectively. (3) April data for (2) with addition of vitamin D. (4) April data for (1) with addition of vitamin D.

In the present experiment the diet containing 0·28 per cent. Ca (group 2) produced little change in the concentration of the blood constituents. In the previous winter two animals out of ten fed this diet showed low serum Ca values at slaughter. The blood phosphatase results for group 2 are interesting; at slaughter they were 50 per cent. lower than those obtained for animals in any other group. The group average of 3·8 units per 100 c.c. serum at slaughter is particularly noticeable. This point will be returned to later.

With regard to the two treatments with the next highest levels of Ca no great difference in the concentration of the blood constituents is apparent. The ration containing 0·86 per cent. Ca, however, tended to produce hypercalcaemic values in some instances which accounts for the higher blood Ca of this group. The serum phosphatase of the two groups is alike apart from the values at the second sampling; nevertheless, the concentration of the enzyme is higher in both groups than in the group considered just previously.

Animals on the highest level of Ca developed a blood picture of hypercalcaemia and hypophosphataemia which gradually became more marked. Two animals were still making normal growth at bacon weights with serum Ca values of 14·2 and 15·7 mg. respectively. The high phosphatase figures, however, show that their metabolism was upset. The condition of two other animals necessitated a change of diet, and they were fed the ration containing 0·09 per cent. Ca. At the next blood sampling they had developed a blood picture similar to group 1 (Table XVI (2)). When vitamin D was now added to the diet the blood picture returned to normal (Table XVI (3)).

Bone analyses.

The treatments which produced poor growth rate and appetite and gave rise to an abnormal blood picture were also characterised by their adverse effect on the chemical composition of the bone. The highest and lowest levels of Ca definitely lowered the amount of ash in the fat-free bone. The tibias of some of these animals were bent and deformed. On the whole the bones of the animals on the 0·28 per cent. and the 0·86 per cent. levels of Ca showed normal values, but in one instance in each group an abnormally low ash content was found. The medium level of Ca alone produced normal bones of high ash content in every case.

Table XVII. *Bone analyses, Exp. 5. Percentage of ash in the dry fat-free tibia obtained from the left hind limb of each animal at slaughter.*

Level of Ca (%) .	0·09	0·28	0·47	0·86	1·62
Ca/P ratio .	1/6·3	1/2·0	1/1·18	1/0·64	1/0·34
	53·6	57·1	56·7	52·2	54·3
	53·2	56·7	57·9	57·1	53·6
	54·8	56·9	57·3	57·2	55·4
	—	54·2	56·3	57·2	56·8
	—	57·8	58·2	56·6	53·6
	—	—	56·6	56·8	55·7
	—	—	57·7	56·8	—
Av.	53·9	56·5	57·2	56·3	54·9

SUMMARY OF THE EXPERIMENTAL RESULTS.

In the final assessment of the suitability of a ration for producing a normal physiological state in the animals, the different aspects studied in these investigations have been considered together. From the aspect of growth as determined by live-weight increase it has been shown in every experiment that the Ca content of the ration has no influence on the utilisation of the food. Definite differences between treatments, however, were obtained with respect to growth and appetite, or ability to maintain a given plane of nutrition, and the suitability of the ration for growth has been judged on this basis. Rations which have given rise to an abnormal concentration of any constituent in the blood are considered unsuitable. From the aspect of bone development an ash content of less than 55 per cent. in the dry fat-free bone was assumed to be the result of an unsuitable dietary. In Table XVIII the experiments have been summarised in this manner to show at a glance the nature of the physiological state produced in the animals. If the diet has given rise to an abnormal condition in relation to growth, the blood picture, or composition of the bone it must necessarily be considered as being unsuited for the true requirements of the animal.

The data in Table XVIII are presented in graphical form in Fig. 1, in which the reaction of the animals to rations of differing Ca and P content is suitably indicated. The results of other workers have been treated in the same manner as in this investigation, and their findings are included in the figure along with the writer's.

It will be seen that surprisingly good agreement has been obtained at the various centres in so far as there is a sharp distinction between Ca and P levels which give rise to a normal physiological state and those which do not. There are two notable exceptions, however. Spildo's level of 0·5 per cent. CaO and CaO/P_2O_5 ratio of 1/2 (15) which he considers as optimal for swine is seen to be somewhat low. The rations he used, however, contained whole dried milk which is usually more potent in vitamin D than cereal by-products and plant-protein meals, and thus a lower level of Ca would suffice. Nevertheless, the low values obtained for blood phosphatase in animals fed a diet of similar Ca/P ratio (Exp. 5) would seem to show that the ratio advocated by Spildo approximates to the optimal.

Table XVIII. *Summary of experiments.*

	Ration				Effect on animals			
Experiment	Ca %	P %	Ca/P	Vitamin D I.U.	Growth	Blood picture	Bones	General effect
1 (winter)	0·29	0·57	1/1·95	—	N.	Ab.	—	Ab.
	0·29	0·57	1/1·95	3000*	N.	N.	—	N.
2 (summer)	0·15	0·53	1/3·7	—	Ab.	Ab.	Ab.	Ab.
	0·15	0·53	1/3·7	1500*	N.	Ab.	Ab.	Ab.
	0·15	0·53	1/3·7	6000*	N.	Ab.	Ab.	Ab.
	0·34	0·53	1/1·6	—	N.	N.	N.	N.
	0·34	0·53	1/1·6	1500*	N.	N.	N.	N.
	0·34	0·53	1/1·6	6000*	N.	N.	N.	N.
3 (winter)	0·19	0·57	1/3·0	—	Ab.	Ab.	Ab.	Ab.
	0·19	0·57	1/3·0	900†	Ab.	?	?	?
	0·19	0·57	1/3·0	1800†	Ab.	?	?	?
	0·32	0·57	1/1·8	—	N.	N.	Ab.	Ab.
	0·28	0·57	1/2·0	—	N.	Ab.	Ab.	Ab.
	0·29	0·57	1/2·0	—	N.	N.	Ab.	Ab.
4 (summer)	0·86	0·56	1/0·65	—	N.	N.	N.	N.
	0·86	0·56	1/0·65	900†	N.	N.	N.	N.
	0·86	0·56	1/0·65	2100†	N.	N.	N.	N.
	1·62	0·55	1/0·34	—	N.	Ab.	Ab.	Ab.
	1·62	0·55	1/0·34	4500†	N.	Ab.	Ab.	Ab.
	0·79	0·96	1/1·2	—	N.	N.	N.	N.
5 (winter)	0·09	0·57	1/6·3	—	Ab.	Ab.	Ab.	Ab.
	0·29	0·57	1/2·0	—	Ab.	N.	N.	Ab.
	0·47	0·57	1/1·2	—	N.	N.	N.	N.
	0·86	0·56	1/0·65	—	N.	N.	N.	N.
	1·62	0·55	1/0·34	—	Ab.	Ab.	Ab.	Ab.

a cod-liver oil. † From ostelin. N. = nor Ab. = abnormal.

The second marked discrepancy in the figure is the symbol showing normality at the 1 per cent. CaO level and CaO/P_2O_5 ratio of 1/0·83 which Reimers and Smuts (12) suggest as the proper Ca and P levels for the nutrition of swine. Some difficulty has been experienced, however, in understanding the method they used in calculating the phosphate content of their rations. Table VIII of their paper shows that none of their feeding stuffs contained more than 0·75 per cent. of "phosphorus as P_2O_5," yet the basal ration, comprising these foodstuffs, contained 0·82 per cent. The actual P_2O_5 analyses of their foodstuffs are, also, unusually low. Granted that P_2O_5 deficiency does exist in South Africa and may affect the ash content of cereals grown there, nevertheless, the normal CaO (11·4 per cent.) and low P_2O_5 content (2·4 per cent.) of their fish meal cannot be explained on this basis. Furthermore an analysis of their rations shows that the limestone used as a Ca supplement contained

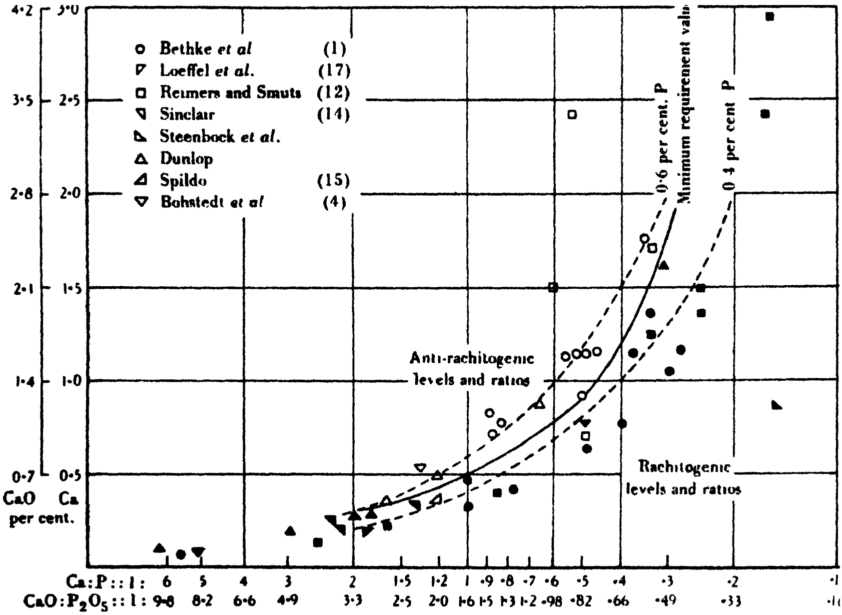

FIG. 1. THE REQUIREMENT CHART—SHOWING THE Ca, P AND VITAMIN D REQUIREMENTS OF SWINE.

In this chart the results obtained in this investigation are presented in tabular form along with the results obtained by other workers in similar investigations.

Along the abscissae are plotted the Ca/P (or CaO/P_2O_5) ratios of the diets. The ordinates show the percentage of Ca (or CaO) in the ration. The solid symbols indicate that the diet was found unsuitable for producing a normal physiological state in the animals. The symbols in outline show that the diet gave normal health and growth.

There is a clear line of demarcation between the rachitogenic and anti-rachitogenic diets. All points above and to the left of this line indicate that these diets have been found suitable without addition of vitamin D for growth rates of 1·0–1·4 lb. per day when the economy of gain varies from 3·0 to 4·0 lb. of dry matter per lb. of live-weight gain.

The vitamin D requirement, above the amount present in the basal diet, is nil in the anti-rachitogenic area of the chart. In the rachitogenic area the requirement is increased the further the point indicating the level and ratio is removed from the minimum requirement line.

only 45–47 per cent. CaO, which besides being an undesirable grade of purity for such experiments may have contained a comparatively high proportion of Mg.

An interesting point that the figure demonstrates is the importance of the Ca/P ratio in animal nutrition. A Ca level adequate at one ratio of Ca/P may be inadequate at another. Thus a Ca level of 0·5 per cent. and Ca/P ratio of 1/1·2 appears to be correct, but if the Ca/P ratio is changed to 1/1·0 this level of Ca is inadequate. Similarly a Ca level of 1·16 per cent. with a ratio of 1/0·47 gives a different picture when compared with the same level at a ratio of 1/0·37. The data in the figure also support the findings of Shohl *et al.* (13), who found that when the level of both Ca and P is raised or lowered, the ratio being kept constant, the rickets-producing quality of the diet is diminished or increased respectively.

It is interesting to note, however, that the line of demarcation between adequate and inadequate ratios of Ca and P follows fairly closely the 0·6 per cent. P level. Bethke *et al.* (1) assert that this level represents the minimal requirement of swine for P, irrespective of the amount of Ca present. But in view of the recent work of Bruce and Callow (5) on the properties of phytin P in cereals, it appears too early to be dogmatic in this respect. According to these workers the optimum level of P should vary according to the cereal composition in the diet. The results obtained at different centres show that such is the case, for in Exp. 2 normality was obtained with only 0·53 per cent. P—a level also found to be sufficient by Sinclair (14), while in the rations of Bethke *et al.* the P level required to be raised to at least 0·6 per cent. for normal growth.

Finally, a rough estimate of the amount of vitamin D required to produce a normal physiological state may be arrived at from a study of the figure. Within the normal area none is required in addition to the amount which may be present in the cereal and protein meals of the basal ration. At other levels and ratios the amount to be added will be proportional to the distance of the point in the figure, representing the particular level and ratio, from the line of demarcation between the suitable and unsuitable diets. It should be remembered, however, that some levels and ratios may be grossly inadequate. A simple calculation will show that no amount of vitamin D will give normal growth (1·2 lb. live-weight increase per day) when added to diets containing less than 0·25 per cent. Ca or 0·35 per cent. P, even assuming complete retention and utilisation.

SUMMARY AND CONCLUSIONS.

The results of experiments conducted on 156 swine, from time of weaning till bacon weights were reached, are reported. One hundred and twenty of these animals were subjected to complete dietary control as regards the quantity of food consumed and the composition of the diet.

Twenty-nine treatments, consisting of different amounts of Ca, P and vitamin D added to an otherwise adequate diet, were imposed.

The reaction of the animals to the treatments was studied in relation to growth (increase in weight), appetite, utilisation of the food, the blood picture (concentration of serum Ca, blood inorganic phosphate, serum phosphatase and blood Hb), and the morphology and chemical composition of the bone. The results obtained are summarised in Table XVIII.

The different levels of Ca and the Ca/P ratios which produced either a normal or an abnormal physiological state in the animals have been plotted out in graphical form along with the results, similarly treated, of investigations by other workers. Fig. 1 shows at a glance those levels of Ca and P in the diet which are optimum for the nutrition of swine. From the figure some indication is also obtained of the amounts of vitamin D which must be added to a rachitogenic diet to give a normal growth and bone formation.

The blood phosphatase was found to be lowest in concentration when the Ca/P ratio in the diet was 1/2.

Some evidence has been adduced that the make up of the cereal portion of the ration exercises some effect on the P requirement of the animals. This is ascribed to the varying amount of phytin P in different cereals.

While the true requirement of an animal for Ca and P must necessarily be dependent on the rate of growth and the economy of gain, the results of this investigation indicate that a diet with a Ca level of 0·45 per cent. of the dry matter and a Ca/P ratio of 1/1·3 is optimal when, as in practice, the average daily rate of growth between the live weights of 30 and 200 lb. varies from 1·0 to 1·4 lb. and the economy of gain from 3·0 to 4·0 lb. of dry matter per lb. of live-weight increase.

My thanks are due to Professor Sir Gowland Hopkins for his continued interest in the work; to Mr A. L. Bacharach of the Glaxo Research Laboratories for generous gifts of ostelin; and to Mr E. A. Porter who was in charge of the experimental animals.

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(*Received October* 1*st*, 1934.)

NUTRITIVE VALUE OF LUCERNE.

III. THE COMPOSITION, DIGESTIBILITY AND NUTRITIVE VALUE OF LUCERNE HAY, LUCERNE MEAL (ENGLISH AND AMERICAN) AND LUCERNE LEAF MEAL (AMERICAN).

By H. E. WOODMAN, M.A., Ph.D., D.Sc.
and A. EDEN, B.A., Dip. Agric. (Cantab.).

(*The Animal Nutrition Research Institute, School of Agriculture, Cambridge University.*)

Introduction.

In a previous communication (1) an account was given of an investigation, carried out during the season of 1932, into the changes of composition, both organic and inorganic, which the lucerne crop undergoes as it passes through the different stages of growth up to flowering. This was followed by a second contribution (2) to the study of the nutritive characters of the lucerne crop, in which was outlined the results of an investigation into the influence of systematic cutting at three different stages of growth, namely, pre-budding, budding and flowering, on the yield, composition and nutritive value of the crop (season 1933).

The combined results of these two investigations furnish all the information necessary to enable the farmer to assess the feeding value of lucerne when cut at any particular stage of growth for feeding to live stock in the fresh, green condition. Lucerne, however, is grown not merely for cutting under the soiling system, but also for conversion into hay for the purposes of winter keep. Further, the practice of drying lucerne by artificial means and grinding the dried product to a meal has been a recognised industrial process in the United States for some years past. Lucerne meal made in this way has acquired considerable popularity as an ingredient of poultry foods, and fairly large amounts have been imported for this purpose into Great Britain. It is only quite recently that attempts to establish the practice in this country have been made. An offshoot of the lucerne-drying industry is the manufacture of lucerne leaf meal, a more valuable feeding product than the meal from the whole plant, since in its production the coarse, fibrous stems of the plant are excluded. Since the leaf meal is not as yet manufactured in this country, the writers were obliged to secure a consignment of this commodity from the United States for the purposes of the present investigation. In this inquiry, the composition and digestibility of the following lucerne products have been investigated:

(1) Lucerne hay made by baling direct from the field.
(2) Lucerne hay made in the stack.
(3) Lucerne meal (English).
(4) Lucerne meal (American).
(5) Lucerne leaf meal (American).

I. COMPOSITION AND NUTRITIVE VALUE OF LUCERNE HAY.

The first sample of baled hay was made from the first cut of a one-year-old stand of lucerne (variety Provence) growing on a light, gravelly soil on the Howe Hill University Farm at Cambridge (season 1933). The crop was cut on May 27, at which date it was in bud and was becoming slightly "laid." The mowing machine on the turns tended to strip the lucerne plants of foliage, leaving the stems of such plants uncut. The crop was converted into hay by the customary procedure, the weather conditions throughout being fine, warm and sunny. It was carted on June 3 and baled on the following day. The bales, which averaged about 120 lb. in weight, were stored in a Dutch barn until the commencement of the digestion trials in the following October.

At the time of cutting the crop for hay, the yield of lucerne was assessed by *scything* and weighing the produce from five plots, each measuring 560 sq. links and situated in different parts of the field. The yield of the crop amounted to 4014 lb. of dry matter per acre. In addition, the total weight of the bales and the moisture content of the lucerne as baled were determined. In this way, it was found that only 3275 lb. of dry matter per acre was actually carted from the field for baling, 18·4 per cent. of the dry matter of the crop thus being unaccounted for. This figure constitutes a guide to the losses incidental to (1) the rather inefficient cutting of the slightly "laid" crop by the horse-mower (see previous paragraph), and (2) the respiration and mechanical losses sustained in the hay-making processes.

The average moisture content of the lucerne as fed into the baler was, despite the hot, dry weather of the hay-making period, as high as 20·7 per cent., this being due to the slowness of desiccation of the lucerne stems. Previous experience, however, had demonstrated that forage of this moisture content could be stored successfully in bales, although its conservation in the stack might result in considerable moulding. This proved to be the case when the bales were opened in October. The mean moisture content of the hay from the bales was 16·5 per cent., this representing an average reduction during storage of about 4 per cent. The partial drying-out of the bales was encouraged by stacking them in the Dutch barn so that air could circulate freely between them. But although only traces of mould development could be noted here and there, the quality of the hay was not very satisfactory. It lacked the attractive colour and smell usually associated with lucerne hay made in the stack.

A further insight into the factors controlling the quality of hay made by direct baling from the field was gained when the second cut of the crop, this time at the early flowering stage, was converted into hay. Cutting took place on July 12. The weather during the first half of the hay-making period was stormy, but the latter half was hot and sunny. The crop was carted for baling on July 20, the mean moisture content being as high as 25·9 per cent. The weight of crop carted from the field amounted to 2000 lb. of dry matter per acre. Unfortunately, after making the first four bales, the operations were affected by the development of weakness in one of the piston castings of the baling machine and the hay was less efficiently pushed out. This led to much more material being compressed into each bale than usual, the subsequent bales averaging about 170 lb. in weight as against about 110 lb. in the case of the four normally compressed bales.

When the bales were opened in the following November, the hay from the first four bales was of good quality and free from mould. Its moisture content had undergone reduction from 25·9 to 15·7 per cent. during storage. The loss of dry matter as a consequence of fermentative action in the bale amounted to no more than 7·5 per cent. of the dry matter originally baled. The hay from these normal bales was used for the purpose of the digestion trials.

The hay from every one of the abnormally tightly packed bales was so spoilt by extensive mould development as to be unfit for consumption by farm animals. The mouldy material averaged 18·7 per cent. of moisture as against 15·7 per cent. in the case of the unspoilt hay. The degree of compression obviously affects the rate of drying-out during storage. When the compression is such as to retard this drying-out process, there is danger of mould growth and spoiling, the risk clearly being greatest when the farmer is taking advantage of the finding that hay may be baled from the field in a somewhat moister condition than would be safe if the material were being filled into a stack. In such circumstances, it is necessary to avoid an abnormal compression of the bales. It is also advisable to stack them up under good cover and in such a manner as to permit of free circulation of air between them.

For purposes of comparison, investigations were also carried out on lucerne hay that had been made in the stack from a one-year-old crop (variety Provence) growing on heavy "skirt" land at Willingham, Cambs. It represented the second cut, in half-flower, taken in early August, 1932. It was made and stacked during a period of hot, sunny weather. The stack was not opened for more than a year (November, 1933), and the hay proved to be of excellent quality. In respect of colour, smell and leafiness, it was far superior to the samples of hay that had been made in the bales.

The data for the composition of the three types of lucerne hay are recorded in Table I. Corresponding data for the composition of the green lucerne from which the hay was made are also given for comparison.

Table I. *Composition of lucerne hay and green lucerne.*

	Lucerne hay	Green lucerne	Lucerne hay	Green lucerne	Lucerne hay	Green lucerne
Site of crop	Howe Hill	Howe Hill	Howe Hill	Howe Hill	Willingham	Willingham
Season	1933	1933	1933	1933	1932	1932
Method of conservation...	Bale	—	Bale	—	Stack	—
No. of cut	1	1	2	2	2	2
Stage of growth	Bud	Bud	Flower	Flower	Flower	Flower
	%	%	%	%	%	%
Moisture content	16·50	78·82	15·70	75·47	13·47	77·40
Composition on basis of dry matter:						
Crude protein	21·09	19·11	16·13	19·53	22·47	21·28
Ether extract	1·11	2·56	1·28	2·98	1·28	2·80
N-free extractives	35·32	39·29	34·40	37·51	36·43	36·11
Crude fibre	31·05	28·12	38·06	29·17	30·24	30·75
Ash	11·43	10·92	10·13	10·81	9·58	9·06
SiO_2-free ash	10·99	10·77	9·79	10·65	9·28	8·94
Lime (CaO)	2·94	3·19	2·83	3·34	3·26	3·17
Phosphoric acid (P_2O_5)	0·85	0·66	0·52	0·66	0·59	0·66
Chlorine (Cl_2)	0·47	0·44	0·49	0·51	0·39	0·30
True protein	15·16	14·52	12·01	15·86	17·58	16·35
"Amides"	5·93	4·59	4·12	3·67	4·89	4·93

Comments on Table I.

It is generally recognised that lucerne is not an easy crop to make into hay. This is due to two reasons: (1) the thick lucerne stems do not dry out very readily; (2) the foliage of the crop becomes brittle in the curing process and easily breaks off unless the dried forage is handled with care. In this way much of the most valuable part of the crop may be lost.

That it is possible, when the requisite conditions are realised, to convert lucerne into hay with little or no deterioration is clearly demonstrated by the data for the Willingham stack hay and the green lucerne from which it was made. Having regard to the difficulties associated with the accurate sampling of crops, the correspondence in this case between the data for the hay and the green lucerne, both in respect of organic and inorganic constituents, is remarkably close. The only really significant distinction is the lower percentage of ether extract in the hay, and judging from the figures for the other samples, this lowering of the content of ether extract appears to be an inevitable consequence of exposing the forage to sunlight in the field-drying stage of the hay-making operations. This has further been proved by determining the ether extract in lucerne immediately after cutting and again at the time when the forage was ready for carting to the stack.

In the case of the Willingham trial, the crop at the time of the second cut in half-flower was remarkably green and leafy; the hay-making was attended by the most favourable weather conditions, and the operations were carried out by a farmer who devoted the utmost care to securing the forage with as little loss as possible. It may be assumed, therefore, that the data for the Willingham stack hay represent the composition of lucerne hay (cut at flowering) at its best. On the other hand, the data for the Howe Hill bale hay (second cut in flower) show pronounced differences from the figures for the green lucerne. The protein content has fallen appreciably, whereas the fibre content has risen from 29·2 per cent. of the dry matter to the very high value of 38·1 per cent. Such modifications of composition are consistent with a considerable loss of the leafy part of the crop during the field operations.

The results for the other sample of Howe Hill bale hay (first cut in bud) are not so easy to interpret, the differences in protein and fibre content between the hay and the green crop not being such as would have been anticipated from the observation, at the time of carting and baling the forage, that a considerable loss of leafage had been sustained. The problem in this instance, however, was complicated by the inefficient cutting of the somewhat "laid" crop by the horse-mower, to which reference has already been made. This meant that large amounts of stem, as well as leaf, were left behind in the field.

Taking into account the widely differing conditions under which the forage in the three separate trials was made into hay, it would manifestly be unsafe to use the data in Table I to assess the comparative merits of stacking and baling as a means of conserving forage in the form of hay.

Digestion trials were carried out on all three types of lucerne hay. Two pure-bred Suffolk wethers, aged about 2½ years, were employed for this purpose, the daily ration in all the periods of feeding consisting of 1400 gm. of the chaffed hay. The results (mean for two sheep) of these trials are summarised in Table II. Unfortunately, no corresponding data for the green lucerne from which the hay was made are available, and for comparative purposes it is necessary to employ results obtained in the investigation of the Willingham crop during 1933. For the full details of the digestion trials, the reader is referred to the appendix to this paper.

Table II. *Digestibility and nutritive value of lucerne hay.*

	Lucerne hay			Green lucerne (2)	
Site of crop	Howe Hill	Howe Hill	Willingham	Willingham	Willingham
Season	1933	1933	1932	1933	1933
Method of conservation ...	Bale	Bale	Stack	—	—
No. of cut	1	2	2	1	2
Stage of growth	Bud	Flower	Flower	Bud	Flower
	%	%	%	%	%
Moisture content as fed	15·90	13·96	13·47	78·26	74·44
Digestion coefficients:					
Organic matter	62·4	54·1	61·1	67·3	64·4
Crude protein	74·3	67·4	73·7	79·8	79·4
Ether extract*	—	13·2	—	20·4	19·0
N-free extractives	67·7	59·9	66·8	75·6	73·7
Crude fibre	50·9	44·6	48·0	49·6	45·4
Digestible composition and nutritive value (dry-matter basis):					
Digestible crude protein	15·67	10·87	16·56	16·48	15·33
Digestible ether extract	—	0·17	—	0·45	0·47
Digestible N-free extractives	23·91	20·61	24·34	30·79	29·41
Digestible fibre	15·80	16·97	14·52	13·89	13·08
Digestible organic matter	55·38	48·62	55·42	61·61	58·29
Starch equivalent†	43·72	31·26	44·27	51·51	47·28
Nutritive ratio	2·53	3·49	2·35	2·77	2·84

* The failure to secure data for the digestion coefficients of the ether extract is not surprising in view of the very low percentage of this constituent in the lucerne hay. In cases such as this, it is commonly found that the output of metabolic ether-soluble material in the faeces is greater than the amount of ether extract in the ration.

† The factor 0·58 has been employed in the correction, on the basis of fibre content, of the starch equivalents of the hay samples. The values so obtained have been increased by one-fifth in accordance with the procedure adopted by T. B. Wood in the case of the coarse fodders (3).

Comments on Table II.

The high quality of the Willingham stack hay, to which attention has already been drawn, is further emphasised by the data in Table II. Although made from a flowering lucerne crop, it is equal, in respect of digestibility and nutritive value, to the Howe Hill bale hay made from lucerne in the bud stage of growth. Nevertheless, a comparison of the data for the Willingham hay with those for green lucerne in bloom suggests that no matter how successfully the conservation is accomplished, there is likely to be a slight, all-round reduction in digestibility as a consequence of the changes that accompany the process. Only in the case of the fibrous constituent is this reduction of digestibility not to be noted; here a slight increase occurred, a finding in harmony with the general observation that the changes in the stack lead to a definite increase in the digestibility of the fibre of a hay crop.

That the diminution in digestibility and feeding value may be very considerable when the necessary conditions for successful conservation are not realised is shown by the data for the Howe Hill bale hay (second cut in flower). In this case, mainly as a consequence of loss of leafage during hay-making, the decrease in digestibility was very pronounced, the digestion coefficient of the organic matter being only 54·1 per cent., as compared with 64·4 per cent. in the comparable green crop. The percentage of digestible protein, on the basis of dry matter, was only 10·9 per cent. (as against 15·3 per cent. in the green lucerne), whilst the starch equivalent was as low as 31·3 (as against 47·3 for the green crop).

It is of interest to compare the results for the Willingham hay with corresponding data for good meadow hay. The comparison is set out in Table III.

Table III. *Comparison of nutritive value of good grades of lucerne hay and meadow hay (dry-matter basis).*

	Good lucerne hay (2nd cut in bloom) %	Good meadow hay %
Crude fibre	30·2	25·8
Starch equivalent	44·3	45·2
Digestible protein	16·6	8·7
Lime	3·3	1·2
Phosphoric acid	0·6	0·5
Chlorine	0·4	0·4

It will be noted from Table III that a very good grade of lucerne hay, made from a crop in flower, has a starch equivalent almost the same as that of a very good grade of meadow hay. It tends to be more fibrous, however, but is distinguished by being nearly twice as rich in digestible protein. There is little to choose between meadow hay and lucerne hay so far as chlorine and phosphoric acid are concerned, the main distinction in respect of mineral composition being the lime content. The lucerne hay contained nearly three times as much lime as the meadow hay, its richness in this constituent being such that 1 lb. of the hay (13·5 per cent. moisture) supplied nearly ½ oz. of CaO.

If it be assumed that a 10 cwt. dairy cow can deal with a daily ration of hay containing 25 lb. of dry matter, then such an allowance of the lucerne hay under consideration would furnish 11·08 lb. of starch equivalent, including 4·15 lb. of digestible protein. The starch equivalent would suffice for maintenance and 1·8 gallons of milk, whilst the digestible protein would cover the needs of maintenance and the production of 5·7 gallons (3·7 per cent. fat). Incidentally, the lime supply in the ration, 13·2 oz., would meet the demands of maintenance and the production of about 23 gallons of milk. It is clear from the foregoing considerations that such lucerne hay, from the standpoint of starch equivalent supply, should not form the sole diet of cows yielding more than 1½ gallons of milk. For higher-yielding animals, the hay allowance should be cut down and fed in conjunction with a concentrated supplement. It follows from the richness of the hay in lime and digestible protein, that the cereals, with their low lime and digestible protein content, may be used more liberally in such supplements than is the case when the coarse fodder of the ration is supplied in the form of meadow hay.

II. COMPOSITION AND NUTRITIVE VALUE OF LUCERNE MEAL AND LUCERNE LEAF MEAL.

In recent years lucerne meal has acquired considerable popularity in this country. It has commanded comparatively high prices, its reputation being in some measure due to statements respecting its content of plant pigments and accessory food factors. In the account which follows, however, attention will be focussed solely on its value as a source of starch equivalent and digestible protein.

Three samples of lucerne meal were investigated in these experiments, two of which were made in this country, the third having been imported, for comparative purposes, from the United States. The procedure adopted in the manufacture of the English lucerne meal is as follows: the crop is cut, usually before flowering, with a tractor-driven cutter. The forage is then loaded into lorries and transported to the drying plant. It is there unloaded and cut into short lengths in an ensilage cutter, which blows it direct into the drying drum. This drum is about 40 ft. long, and the cut crop is subjected to a hot blast while travelling through. The maximum temperature of the blast is in the region of 700° F., but the heat supply is so regulated as to avoid scorching of the issuing dry material. The latter is subsequently fed through a grinder and thence into bags.

The first sample of English lucerne meal was made from a first cut of lucerne in the pre-budding stage. At the time of its reception from the factory (May 16, 1933), the fine, green meal contained no more than 7·14 per cent. of moisture. The sacks containing it were stored in a cool, dry place until the date of the digestion trial in the following December, at which time the moisture content was found to have risen to 10·57 per cent. No deterioration in quality had occurred during storage.

The second English lucerne meal sample was manufactured from a second cut of the crop in very early flower. It contained 9·56 per cent. of moisture when received from the factory (July 13, 1933), and at the time of the digestion trial in January 1934, the value had risen to 9·75 per cent. Again, no deterioration in quality was noted as a result of storage.

It will be noted that the English lucerne meal was manufactured by artificially drying the green crop, a process that was first practised on an industrial scale by Mason in the United States. In the southerly regions of the United States, however, it is possible to dispense with artificial heat by utilising the heat of the sun for this purpose. In other words, the lucerne is first converted into hay and then ground to a meal to be sold under the name of alfalfa meal. Such was the origin of the sample of American lucerne meal employed in the present investigations. It came from New Mexico, U.S.A., and despite its long land and ocean transport, it contained no more than 8·23 per cent. of moisture on arrival. It may be noted that lucerne meal of this character is imported regularly into this country.

A modern development of the lucerne-drying industry in America is the production of lucerne *leaf* meal, in which practically all the leaves and only a small proportion of the stems are included. A sample of this grade was received at the same time as the American lucerne meal. It showed no signs of deterioration as a result of long transport and contained only 8·48 per cent. of moisture. The details of its manufacture are as follows: the whole lucerne hay is first ground through a comparatively coarse screen, with perforations usually about ¼ in. in diameter. The resulting product is then run through a sifting device which is provided with a very fine mesh wire screening cloth, usually 34 mesh. The leaves, which pulverise readily in the first grinding, bolt through the sifting cloth, while the stems pass on over it. Thus the material passing through the cloth is the leaf meal and that passing over is the stem meal. It is of interest to record that the *wholesale* prices in December 1933 were about £9 per ton for the leaf meal and £7. 10*s*. per ton for the whole lucerne meal.

In Table IV are summarised the data for the composition of the samples of lucerne meal and lucerne leaf meal. For comparative purposes, the composition of green lucerne in the budding and early flowering stages is also shown.

Table IV. *Composition of lucerne meal and lucerne leaf meal (dry matter basis).*

	English lucerne meal (1st cut in pre-bud)	English lucerne meal (2nd cut in early flower)	American lucerne meal	American lucerne leaf meal	Green lucerne (2)	
					(1st cut in pre-bud)	(2nd cut in early flower)
	%	%	%	%	%	%
Crude protein	24·45	17·82	13·27	23·53	25·33	19·31
Ether extract	3·19	2·64	1·28	2·06	2·35	2·47
N-free extractives	39·98	41·40	39·76	44·62	38·13	39·91
Crude fibre	19·82	26·93	36·26	17·59	22·12	28·82
Ash	12·56	11·21	9·43	12·20	12·07	9·49
SiO_2-free ash	9·06	10·14	9·11	10·32	11·21	9·43
Lime	2·90	3·98	1·75	2·19	2·98	3·64
Phosphoric acid	0·67	0·45	0·43	0·47	0·88	0·57
Chlorine	0·61	0·52	0·73	1·04	0·36	0·25
True protein	19·29	15·48	10·64	21·94	18·24	14·93
"Amides"	5·16	2·34	2·63	1·59	7·09	4·38

Comments on Table IV.

It should first be pointed out that the comparisons in Table IV are necessarily of a somewhat tentative character, since climate, soil conditions and manurial factors exert an influence on the composition of lucerne, particularly on the mineral composition, that makes comparison of crops grown in differing localities not always a straightforward matter (1). For really significant comparison, it would be desirable that all the samples of lucerne meal should have been manufactured from the same lucerne crop. Although, for obvious reasons, such ideal conditions could not be realised in these trials, it is nevertheless possible to draw attention to several features of interest.

It has already been shown that hay-making leads to a pronounced reduction in the amount of ether extract in lucerne. The figures for the English lucerne meal indicate that this loss may be avoided when the green crop is dried artificially. Not only is the colour of the lucerne retained almost unimpaired, but the actual amount of ether extract suffers little or no diminution in the drying process. The percentage of ether extract in the American lucerne meal is low, but this was to be expected in view of the fact that the meal in this case was made by grinding lucerne hay instead of the artificially dried crop.

The process of drying lucerne artificially has a very great advantage over hay-making in that the green crop can be collected for transport to the drying appliances without having sustained the mechanical losses, chiefly in the form of leaf, that usually occur when the crop is converted into hay. Further, the subjection of the green crop to artificial heat immediately after cutting enables the further losses, associated with plant cell respiration, to be avoided. It would be anticipated, therefore, that lucerne meal made in this way would compare much more closely in composition, particularly in respect of protein and fibre content, with the green crop than would lucerne hay.

That this is so is shown by the data in Table IV. Although the figures for the English lucerne meals and the green lucerne are not, for reasons already given, strictly comparable, it is apparent that the data for the organic composition of the meals are such as might equally well be attributed to pre-budding and flowering lucerne in the green condition. It is interesting to note that the percentages of lime and phosphoric acid in the two meals conform with the finding of an earlier investigation (1), namely, that the lime content of lucerne increases as the crop progresses in maturity, whereas the content of phosphoric acid displays the opposite variation. It is also noteworthy that the lucerne crop from which the English meals were manufactured is characterised by a higher chlorine content than the crops from which the data for green lucerne were obtained. This is confirmatory evidence of the influence of local soil conditions in causing modifications of the mineral composition of lucerne. Attention should finally be directed to the high silica content of the pre-budding meal, a result that points to the danger of soil contamination when the crop is cut and collected at a very early stage of growth.

It is an obvious disadvantage to proceed to the manufacture of the meal by means of grinding lucerne hay instead of the artificially dried crop, since if the process of sun-drying in the field should result, as frequently is the case, in the breaking off and loss of leafage, the resulting meal may have a correspondingly poorer composition and feeding value. This possibility is illustrated by the data for the American lucerne meal of the present investigation. Its low protein content (13·3 per cent. of the dry matter) and unusually high fibre content (36·3 per cent.) suggest that the crop was cut at an advanced stage of growth, or, alternatively, that large losses of leaf occurred during sun-drying and collection. The relatively low lime content of the meal points to the latter explanation as being more probable. It is clearly important to know the protein and fibre content of lucerne meal when making purchases of this commodity.

The all-round improvement in composition occasioned by excluding as large a proportion as possible of the tough stems during grinding is revealed by the data for the American lucerne *leaf* meal as compared with those for the American *whole* lucerne meal. The increased protein content (23·5 as against 13·3 per cent. of the dry matter) and greatly reduced content of fibre (17·6 as against 36·3 per cent.) are specially prominent features of this improvement, although this is further manifested in the increased percentages of ether extract, N-free extractives, lime and chlorine. The high percentage of the last-named constituent, both in the leaf meal and the whole meal, appears to be an outstanding characteristic of the American products. Although the composition of the leaf meal, which tends to approximate to that of the whole meal made from pre-budding lucerne, is very satisfactory, there can be little doubt that it would be susceptible of further improvement if the artificially dried crop, instead of lucerne hay, had been employed in its manufacture.

The digestion trials of the lucerne meals were carried out on the same sheep as had been used for the purpose of the lucerne hay experiments. It was first necessary to discover in what form the meal was most acceptable to the animals. Attempts to feed the meal in the dry condition had to be abandoned at the outset, since by no means could the sheep, even when obviously hungry, be induced to consume the dry meal. The food in this state collected around the eyes and the fine, dusty material blew up into the nostrils, producing a distressing and irritating effect. In this connection, it may be pointed out that the mixing of the meal prior to sampling and weighing out the rations for the period was an extremely unpleasant experience for the workers engaged in the investigations.

It was clear that no animal could be expected to deal with the meal in the dry condition. In the next attempts, it was well mixed with its own weight of water. The latter was completely absorbed, giving a moist product with no surplus liquid. The meal in this form was readily and completely consumed by both sheep.

The digestibility of the first sample of English lucerne meal (made from pre-budding lucerne) was determined by this direct method of feeding, the daily ration consisting of 1400 gm. of meal in admixture with 1400 c.c. of water. The trial proceeded quite satisfactorily until the final day, when one of the animals refused obstinately to consume the moistened meal any longer. Neither sheep was observed to ruminate during this period. For the remaining three digestion trials, therefore, the daily ration was altered to 1000 gm. of meal, moistened with 1000 c.c. of water, together with 400 gm. of the chaffed lucerne hay made from the Howe Hill (1933) budding crop, the digestibility of which had been determined in the trials described in the preceding section of this paper. No further difficulties in connection with feeding were then encountered.

The foregoing account of the difficulties experienced in the feeding of the lucerne meal suggests that it is a mistake to reduce the dried lucerne to a fine, dusty meal for use with sheep and cattle. In the feeding of poultry, which are unable to digest fibre beyond a small extent, the grinding of the lucerne is probably of distinct advantage in increasing the availability of the protein, oil and carbohydrate in the feeding product. Also for pigs, the meal supplies the lucerne in a compact form that can readily be incorporated into the ordinary pig slops. But for ruminants, the dried lucerne should not be sold as a meal unless it can be demonstrated that grinding leads to an increase in digestibility. It will be shown, however, that grinding to a meal appears to exercise no such influence in the case of ruminant nutrition, and since the dusty character of the meal introduces difficulties both for the animals and for the stockmen who have to handle the product in bulk, it would seem desirable that this practice should not be followed when the product is intended for sheep and cattle, even though the problem of storage and transport should thereby be rendered somewhat more difficult. From

Table V. *Digestibility and nutritive value of lucerne meal, lucerne leaf meal and green lucerne.*

	English lucerne meal (1st cut in pre-bud)	English lucerne meal (2nd cut in early flower)	American lucerne meal	American lucerne leaf meal	Green lucerne(2)	
					(1st cut in pre-bud)	(2nd cut in early flower)
	%	%	%	%	%	%
Digestion coefficients:						
Organic matter	69·2	63·5	54·8	69·6	75·9	64·4
Crude protein	71·6	71·4	69·4	76·3	84·0	79·4
Ether extract	45·5	28·3	25·1	—	38·0	19·0
N-free extractives	78·0	73·9	67·8	77·6	79·8	73·7
Crude fibre	52·6	45·6	36·4	49·9	63·7	45·4
Digestible composition and nutritive value (dry-matter basis):						
Digestible crude protein	17·51	12·72	9·21	17·95	21·28	15·33
Digestible ether extract	1·45	0·75	0·32	—	0·89	0·47
Digestible N-free extractives	31·18	30·59	26·96	34·63	30·43	29·41
Digestible fibre	10·43	12·28	13·20	8·78	14·09	13·08
Digestible organic matter	60·57	56·34	49·69	61·36	66·69	58·29
Starch equivalent*	55·09	48·45	38·91	55·18	59·81	47·28
Nutritive ratio	2·57	3·51	4·44	2·42	2·19	2·84

* In calculating the starch equivalents of the meals, the fibre correction factor 0·29 has been used.

the farmers' standpoint, it should also be borne in mind that grinding to a dusty meal might, in some instances, enable an inferior, over-fibrous product to escape detection.

In Table V are summarised the results of the digestion trials of the three samples of lucerne meal and the sample of lucerne leaf meal. Comparative data for green lucerne are also given.

Comments on Table V.

From what has been written in a previous publication (2), it is obvious that the digestibility and nutritive value of lucerne meal must depend substantially on the stage of growth at which the crop is cut for drying and grinding. It is therefore in accordance with anticipation that the English lucerne meal made from lucerne at the pre-budding stage of growth should display a better digestibility and higher content of starch equivalent and digestible protein than the meal made from the early flowering crop.

It had been surmised that the process of grinding the dried lucerne to a fine meal might lead to a distinct improvement in digestibility and feeding value, and that the nutritive value of the meal might be significantly higher than would be anticipated from a consideration of the digestibility and starch equivalent of green lucerne. That this is not the case, however, is revealed by comparing the data for the two samples of English lucerne meal with those for green lucerne at the appropriate stages of growth. There is no evidence of any gain in digestibility and nutritive value from drying and grinding, not even in the case of the fibrous constituent. Indeed, the fibre of the pre-budding lucerne meal was poorly digested compared with the fibre in the pre-budding green lucerne, but this may have been caused by the inability of the sheep to ruminate during this first period of feeding, when the meal was fed without the supplement of lucerne hay that was given in the remaining periods. So far as the protein constituent is concerned, the process of drying appears to lead to a distinct reduction of digestibility, a change which is reflected adversely in the digestible protein content of the meal.

The relatively poor digestibility and low content of starch equivalent and digestible protein of the American lucerne meal need occasion no surprise after what has already been written concerning the crude composition of this sample. It has been explained that this meal was made by grinding lucerne after its conversion into hay, and that during the sun-drying process it is probable that appreciable losses of the most highly-digestible portion of the crop, namely, the protein-rich leaf, had occurred. For the uniform production of lucerne meal of highest digestible protein and starch equivalent content, therefore, it is necessary to collect the crop in the green condition and to dry it artificially.

The data in Table V bring out the relatively high digestibility, digestible protein content and starch equivalent of the American lucerne leaf meal. It is noteworthy how closely in all these respects the leaf meal resembles the whole lucerne meal made from the crop at the pre-budding stage. The former, per 100 lb. of dry matter, contained 55·2 lb. of starch equivalent, including 17·95 lb. of digestible protein, as against 55·1 and 17·51 lb. respectively of those constituents in the pre-budding lucerne meal. The two meals are clearly interchangeable in the rations of livestock. They may be regarded as concentrates of rather moderate starch equivalent, but furnishing a generous supply of digestible protein. As an illustration of their concentrate character, it may be shown that 4½ lb. of a mixture of two parts by weight of either of these meals (assuming a moisture content of 8 per cent.) with one part of wheat (or other cereal) would supply the starch equivalent and digestible protein necessary for the production of a gallon of Shorthorn milk. For comparison, it should be kept in mind that only 3½ lb. of the usual milk-production mixtures of oil cake and cereal are required for this purpose.

It is clear, therefore, that two methods are available for the purpose of manufacturing a meal of high feeding value from lucerne: (1) by drying and grinding the forage obtained by cutting at the pre-budding stage of growth; (2) by cutting at the flowering stage and excluding the bulk of the fibrous stem during the grinding of the dried product. The leaf meal manufactured by this method closely resembles, in composition, digestibility and nutritive value, the whole meal made by drying and grinding pre-budding lucerne.

Of these two possible methods, the manufacture of the leaf meal is to be preferred, since in this way it is not necessary to have recourse to the dangerous expedient of cutting the crop at too early a stage of growth. The possible injury to the plant and the consequent shortening of the life of the stand that may follow from constantly cutting the crop at the pre-budding stage have been made clear in a previous communication (2), as also has the further disadvantage that under such a system of cutting, the problem of keeping down infestation by weeds and grasses is rendered much more difficult than when the crop is allowed to grow up to bud or flower.

It may be objected that in the manufacture of the leaf meal from the flowering crop, the rejection of the coarse stemmy material (for which, however, it should not be difficult to find a useful commercial application) leads to uneconomic yields of meal. Against this must be set the finding that systematic cutting at the pre-budding stage also results in much smaller yields than are obtained by cutting at flower. In yield trials carried out during 1933, for example, five pre-budding cuts gave rise to 3967 lb. of dry matter per acre, whereas three flowering cuts from plots on the same field produced as much as 7551 lb. Since the latter was made up of a first cut yielding 4125 lb. of dry matter (leaf : stem ratio = 0·73), a second cut of 2544 lb. (leaf : stem ratio = 1·07) and a third cut of 882 lb. (leaf : stem ratio = 1·28), it follows that if the forage had been used for making lucerne leaf meal, the yield of this product, on the basis of lb. dry matter per acre, would have amounted to 3548 lb. This compares satisfactorily with the yield of whole meal that would have been secured from the five cuts of pre-budding lucerne, particularly when account is taken of the probability that a continuation of the pre-budding system of cutting into the second and third seasons would lead to a much more rapid deterioration in productivity than when the crop is cut continuously in early flower. It may be concluded, therefore, that the manufacture of the leaf meal is one that should receive close attention from those concerns in this country that are interested in crop-drying on a commercial scale.

It now remains to compare the nutritive properties of the various lucerne meals with those of dried grass. Data on which such a comparison may be based are set out in Table VI. It should be stated that the figures for grass are taken from the results of digestion trials of fresh herbage, but the comparison is not on that account weakened, since recent work on grass-drying has shown that grass suffers little or no deterioration of digestibility and nutritive value provided the temperature of drying is suitably regulated.

Table VI. *Comparison of nutritive properties of lucerne meal, lucerne leaf meal and dried grass* (*dry-matter basis*).

	Lucerne meal			Pasture grass	
	Pre-budding crop %	Flowering crop %	Lucerne leaf meal %	Fortnightly (4) cuts %	Monthly (5) cuts %
Digestible protein	17·51	12·72	17·95	18·80	13·00*
Digestible organic matter	60·57	56·34	61·36	72·20	72·50
Starch equivalent	55·09	48·45	55·18	69·90	66·50
Digestion coefficient of crude fibre	52·6	45·6	49·9	81·4	82·2

* Represents digestible protein content of flush and post-flush grass. The fore-flush grass is as rich as the fortnightly cuts in this constituent.

Comments on Table VI.

The data in Table VI leave no doubt as to the possibility of obtaining an artificially-dried product of appreciably higher digestibility and starch equivalent from grass than from lucerne. Pasture herbage at its best has been shown to contain more than 20 per cent. of digestible protein and 70 per cent. of starch equivalent (dry-matter basis). Dried grass cakes made from pasture herbage cut at intervals of a week to a fortnight have been used successfully to replace oil cakes in the winter feeding of dairy cows and fattening bullocks (6). As the intervals between successive cuts is lengthened, the yield of grass is increased, and the concentrate obtained by artificial drying takes on a more suitable "balance." When cut at monthly intervals, for example, the dry matter of the grass contains 66·5 per cent. of starch equivalent, including 13·0 per cent. of digestible protein, and a ration of about 34 lb. of dried grass of this nature, assuming a moisture content of 8–10 per cent., would supply all the requirements of a 5-gallon cow (7).

For a detailed examination of the distinguishing nutritional characteristics of grass and lucerne, the reader should consult a previous communication (2). The early stage of growth at which the fibre in lucerne begins to undergo lignification is reflected in the low digestion coefficients for this constituent in both the samples of lucerne meal and also in the leaf meal. The fibre in the pasture cuts, on the other hand, displays no evidence of lignification, and because of the higher digestibility of grass submitted to systems of cutting at intervals of one to five weeks, the product obtained by artificially drying such pasture cuts has a very distinctly higher starch equivalent not only than lucerne meal, whether made from the flowering or pre-budding crop, but also than the leaf meal. It should finally be noted that under favourable conditions in respect of type of herbage, and of manurial and climatic factors, the yield, in terms of lb. dry matter per acre per season, from pastures under a system of monthly cuts is of the same order as that of lucerne cut systematically at the flowering stage (2).

SUMMARY.

In this paper, which is the third of a series dealing with the nutritive properties of the lucerne crop, an account is given of the results of an investigation into the composition, digestibility and nutritive value of lucerne hay, lucerne meal and lucerne leaf meal.

Three samples of lucerne hay were dealt with, the first two having

been made by baling direct from the field and the third by the usual procedure of filling into the stack. It is shown that by baling from the field, it is possible to conserve forage at a higher moisture content than would be considered safe when filling the material into a stack. In such circumstances, however, too tight a compression of the bales should be avoided, since if the process of partial drying-out during storage be retarded by over-compression, there is danger of extensive mould development and spoiling. It is advisable that the bales should be stored under good cover and in such a manner as to permit of free circulation of air between them. The hay from the bales was of much poorer quality than that made in the stack.

The main difficulties in making lucerne hay are (1) the slowness of drying-out of the thick stems; (2) the tendency of the foliage to become brittle during the curing process and to break off unless the dried forage is handled with care. That this may lead to a very significant fall in protein content and a corresponding rise in the percentage of fibre, as well as a serious decline in digestibility and feeding value, is clearly demonstrated by the results of the present investigation. In all cases, a depression of the percentage of ether extract, as a result of the changes occurring in the crop during sun-drying in the field, was noted.

Even when the ideal conditions for successful conservation are realised, as in the case of the stack hay investigated in these experiments, it is still likely that the hay will display a slightly lower digestibility and feeding value than the green crop from which it was made. Only in the case of the fibrous constituent is any improvement in digestibility to be noted.

It is shown that a good grade of lucerne hay made from a crop in early flower has a starch equivalent almost the same as that of a good grade of meadow hay. It tends to be rather more fibrous, however but is specially distinguished by being nearly twice as rich in digestible protein and almost three times as rich in lime. Illustrations are given in the text of the uses to which such lucerne hay may be put in the feeding of dairy cattle.

Of the three consignments of lucerne meal investigated in these trials, two were made in this country by artificial drying followed by grinding, while the third was made in the United States by sun-drying followed by grinding. The manufacturing processes involved in the production of these meals are described in the text. A sample of American lucerne leaf meal, made by grinding sun-dried lucerne in such a way as to exclude a large proportion of the fibrous stems, was also submitted to investigation.

It is shown that such lucerne meals may be kept over long periods without deterioration or significant increase in moisture content if placed in a cool, dry store. Meals made from artificially-dried lucerne contain as much ether extract as the green crop. The composition of lucerne meals made by this method resembles very closely, on the basis of dry matter, that of the lucerne crops used in their manufacture. Grinding to a meal leads to no increase in the digestibility of the material when fed to sheep and cattle, not even in the case of the fibrous constituent. So far as the crude protein is concerned, the drying process appears to lead to a distinct reduction of digestibility, a change which is reflected adversely in the digestible protein content of the meal.

For the uniform production of lucerne meal of highest digestible protein and starch equivalent content, it is necessary to collect the crop in the green condition and to dry it artificially. The manufacture of lucerne meal by grinding the hay resulting from sun-drying, as in the case of the present sample of American lucerne meal, may involve all the disadvantages associated with the conversion of lucerne into hay. Should large losses of leaf occur in the sun-drying process, a meal of low starch equivalent and digestible protein content may result.

The results emphasise the relatively high digestibility, digestible protein content and starch equivalent of lucerne leaf meal. In all these respects, the leaf meal resembles very closely the whole meal made from lucerne at the pre-budding stage of growth. The two meals are interchangeable in the rations of live-stock and may be regarded as concentrates of rather moderate starch equivalent, but furnishing a generous supply of digestible protein. Reasons are brought forward to show why, of these two possible methods of producing a meal of high feeding value from lucerne, the manufacture of the leaf meal is to be preferred.

The superiority, in respect of digestibility and starch equivalent, of dried grass made from pasturage cut at intervals of one to five weeks over all the forms of lucerne meal and leaf meal is demonstrated from the results of this and previous investigations.

Evidence is brought forward to show that it is undesirable to grind lucerne to a fine, dusty meal when the product is intended for use with sheep and cattle. In the case of pigs and poultry, however, the use of the dried product in the form of meal is quite sound.

In conclusion, the writers gladly take this opportunity of expressing their indebtedness to their colleague, Dr R. E. Evans, for generous assistance in the carrying out of this investigation. Thanks are also tendered to the English and American Companies who kindly supplied the various grades of lucerne meal for the purpose of the comparative trials. Acknowledgments are also due to Messrs V. Thurlbourn and C. Bendall for the care bestowed on the experimental animals during the progress of the digestion trials.

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(*Received October* 1*st*, 1934.)

Digestion tables.

	Sheep XXIV							Sheep XXV						
	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
(1) Lucerne bale hay (1st cut in bud):														
Daily ration	1177·40	1042·82	248·31	13·07	415·86	365 58	134·58	1177·40	1042·82	248·31	13 07	415·86	365·58	134·58
Voided	469·10	395·90	65·50	14 00	134·58	181·82	73·20	459 48	387·36	62·13	13·87	134·03	177·33	72·12
Digested	708·30	646·92	182·81	—	281·28	183·76	61·38	717·92	655·46	186·18	—	281·83	188·25	62·46
Digestion coefficients (%)	60·16	62·04	73·62	—	67·64	50·27	45·61	60·98	62·85	74·98	—	67·77	51·49	46·41
(2) Lucerne bale hay (2nd cut in flower):														
Daily ration	1204·60	1082·57	194·30	15 42	414·38	458·47	122·03	1204·60	1082·57	194·30	15·42	414·38	458·47	122·03
Voided	599·30	521·93	67·66	14·08	176·56	263·63	77·37	540·40	472·04	59·01	12·70	155·91	244·42	68·36
Digested	605·30	560·64	126·64	1·34	237·82	194·84	44·66	664·20	610·53	135·29	2·72	258·47	214·05	53·67
Digestion coefficients (%)	50·25	51·79	65·18	8·69	57·39	42·50	36·60	55·14	56·39	69·63	17·64	62·37	46 69	43·98
(3) Lucerne stack hay (2nd cut in flower):														
Daily ration	1211·40	1095·35	272·20	15·51	441·31	366·33	116·05	1211·40	1095·35	272·20	15·51	441·31	366·33	116 05
Voided	513·02	434·24	73·94	17·77	148·62	193·91	78·78	496·23	418·10	69·00	17·01	144·83	187·26	78·13
Digested	698·38	661·11	198 26	—	292·69	172·42	37 27	715·17	677 25	203·20	—	296·48	179·07	37·92
Digestion coefficients (%)	57·65	60·36	72·84	—	66 32	47 07	32·12	59·04	61·83	74·65	—	67·18	48·88	32·68
(4) English lucerne meal (from 1st cut in pre-budding stage):														
Daily ration	1252·00	1094·75	306·11	39·94	500·55	248·15	157·25	1252·00	1094·75	306·11	39·94	500·55	248·15	157·25
Voided	447·86	329·95	89·50	22 04	104·26	114·15	117·91	462·90	343·43	84 48	21·48	116·28	121·19	119·47
Digested	804·14	764·80	216·61	17 90	396·29	134·00	39·34	789·10	751·32	221·63	18·46	384·27	126·96	37·78
Digestion coefficients (%)	64·23	69·86	70·76	44 82	79·17	54·00	25·02	63·03	68·63	72·40	46·22	76·77	51·16	24·03
(5) English lucerne meal (from 2nd cut in early flower):														
Lucerne meal	902·50	801·33	160·83	23 83	373·63	243 04	101·17	902·50	801·33	160·83	23·83	373·63	243·04	101·17
Lucerne hay	354·00	313·54	74·66	3·93	125·03	109·92	40·46	354·00	313·54	74·66	3·93	125·03	109·92	40·46
Total daily ration	1256·50	1114·87	235·49	27·76	498·66	352·96	141 63	1256·50	1114·87	235·49	27·76	498·66	352·96	141·63
Total voided	479·10	394·46	64·42	19·04	130·94	180·06	84·64	520·45	425·68	65·80	22 97	144·50	192·41	94·77
Total digested	777·40	720·41	171·07	8 72	367·72	172 90	56·99	736·05	689·19	169·69	4·79	354·16	160·55	46·86
Digested from lucerne hay	213·24	194·79	54 96	—	84·57	55·26	18 45	216·09	197·31	55·98	—	84·73	56·60	18·78
Digested from lucerne meal	564·16	525·62	116·11	8·72	283 15	117·64	38·54	519·96	491·88	113 71	4·79	269·43	103·95	28·08
Digestion coefficients of meal (%)	62 51	65·59	72 19	36 59	75·78	48·40	38 09	57·61	61·38	70·70	20 10	72·11	42·77	27·76
(6) American lucerne meal:														
Lucerne meal	917·70	831·17	121·78	11·75	364·88	332·76	86·53	917·70	831·17	121·78	11·75	364·88	332·76	86·53
Lucerne hay	355·40	314·78	74·95	3·95	125·53	110 35	40 62	355·40	314·78	74·95	3·95	125·53	110·35	40·62
Total daily ration	1273·10	1145·95	196·73	15 70	490·41	443·11	127·15	1273·10	1145·95	196·73	15·70	490·41	443·11	127·15
Total voided	552·40	490·48	55·79	12 60	157·66	264 43	61·92	558·00	496·34	57·36	12·89	158·75	267·34	61·66
Total digested	720·70	655·47	140 94	3·10	332 75	178·68	65 23	715 10	649·61	139·37	2·81	331·66	175·77	65·49
Digested from lucerne hay	214·09	195·56	55·18	—	84 91	55 47	18·53	216·94	198·09	56·20	—	85·07	56·82	18·85
Digested from lucerne meal	506·61	459·91	85·76	3·10	247·84	123 21	46·70	498·16	451·52	83·17	2·81	246·59	118·95	46·64
Digestion coefficients of meal (%)	55 20	55·33	70·42	26·38	67·92	37·03	53 97	54·28	54·32	68·29	23·91	67·58	35·75	53·90
(7) American lucerne leaf meal:														
Lucerne leaf meal	915·20	803·54	215·35	18·85	408·36	160·98	111·66	915·20	803·54	215·35	18·85	408·36	160·98	111·66
Lucerne hay	354 90	314·34	74·85	3·94	125·35	110 20	40·56	354·90	314·34	74·85	3·94	125·35	110·20	40·56
Total daily ration	1270·10	1117·88	290 20	22·79	533 71	271·18	152 22	1270·10	1117·88	290 20	22·79	533·71	271·18	152·22
Total voided	449·03	364·30	69·89	25·75	133 86	134·80	84·73	441·28	359·37	70·42	24·60	129·68	134·67	81·91
Total digested	821·07	753·58	220·31	—	399·85	136 38	67·49	828·82	758·51	219·78	—	404·03	136·51	70·31
Digested from lucerne hay	213·78	195·28	55·10	—	84·79	55·39	18·50	216·63	197·81	56·12	—	84·95	56·74	18·82
Digested from lucerne leaf meal	607·29	558·30	165·21	—	315 06	80·99	48·99	612·19	560·70	163·66	—	319·08	79·77	51·49
Digestion coefficients of leaf meal (%)	66·36	69·48	76·71	—	77·15	50·31	43·87	66·89	69·78	76·00	—	78·14	49·55	46·11

AN ANALYSIS OF THE TIME CHANGE IN MILK PRODUCTION IN INDIVIDUAL LACTATIONS[1].

BY MARJORIE GOOCH.

(With Five Text-figures.)

IT is well known to dairymen that the amount of milk that a cow gives during an individual lactation is not uniform, month by month, but changes in a rather characteristic way. Moreover, the course of milk production varies from one lactation to another of a single cow and also differs from cow to cow. In her succeeding lactations, a cow tends to have a higher total milk production (20, 31). This relation has been studied in considerable detail by many authors, but some of the consequences of the change of milk production with time during a single lactation are not so well known. A cow's milk yield usually starts vigorously and gradually diminishes as the lactation is prolonged. It is the purpose of this paper to study this time change during a single lactation in some detail. The data are quite comprehensive and furnish information on several points which are of considerable biological interest.

First, the form of these time changes of milk production during an individual lactation is studied. This trend may be adequately described solely in terms of the initial yield and the rate of decline. The mathematical constants representing these two factors for each lactation are then studied in relation to certain general and biological conditions.

The calendar year and the month in which a lactation began are the general environmental facts which are studied in respect to the constants of the individual time-change curves.

Of the many biological problems connected with milk production, this paper is concerned with the relation of the constants of these time-change curves to the length of time the cow was pregnant during a given lactation; the age of the cow at the beginning of the lactation; the length of the resting period before the lactation; the number of weeks the cow actually gave milk; and the total 8-months' milk yield.

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[1] Paper No. 198 from the Department of Biostatistics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

The writer wishes to express her appreciation to Dr John W. Gowen of the Rockefeller Institute, Princeton, New Jersey, to Dr Raymond Pearl, Department of Biology, School of Hygiene, and especially to Dr Lowell J. Reed of the Department of Biostatistics, and his staff, for suggestions and guidance throughout this study.

In addition to these factors, this material admits of a study of the variability in initial yield and rate of decline of the herd as contrasted to that of individual cows and the extent to which age changes affect this variation.

HISTORICAL BACKGROUND.

As was stated above, milk yield in a particular lactation usually commences high and gradually diminishes more or less slowly. The way this diminution occurs differs with different breeds of cattle. It has long been contended that the reason why some breeds are more profitable milk producers is that they are more persistent in their yields, that is, the milk production does not fall off so rapidly. Various ways of expressing the rate of decline have been proposed. One of the first methods was that used in 1886 by Sturtevant (27). He expressed the falling off of milk yield with time as a percentage decrease from month to month, an idea that has been used with modification by various workers since. Grady (17) in 1917 followed this idea, but he also introduced a somewhat different index number. He considered the first month's yield as the standard and expressed each succeeding month's yield as a percentage difference from the first, so that the percentages increased during the time studied. Several important papers appeared in 1923 which dealt with this phase of the problem. Sanders (23) used the ratio of total lactation yield to maximum daily yield. Correcting this ratio for the month of calving, he got what he called the "shape figure." This method has the merit that each lactation may be described by a single mathematical expression which will be high if the yield is maintained near the maximum yield and low if the yield drops off rapidly. Ellinger (5) uses the ratio between gross production in the second and in the first 10-week sections of the lactation period. McCandlish (19) in 1924 expressed each month as a percentage of the first. In 1926, Turner (29) was still describing the rate of decline as a percentage of the preceding month's production, although he had previously proposed exponential curves for just such a purpose (Brody, Ragsdale and Turner (1)). There these authors used the equation: "$M_t = M_0 e^{-kt}$, where M_t = milk production during any month t; M_0 is the theoretical value of the milk flow at the time of parturition; e and k have the usual meaning." They applied this equation to average yields for several breeds of cattle and considered it evidence of a monomolecular, chemical reaction controlling milk production. Regardless of the chemical interpretation, such an equation does fit observed milk yields for successive months of a lactation probably as well as any simple curve would. Gaines (6, 8, 9) has used this same exponential curve and also more complex ones to express the relation of milk yield with time. Some of this curve fitting he has done graphically by a method which he devised, and some analytically.

The simple percentage methods are easily understood and determined, and the higher the index value the more persistent the cow in milk yield. The indices derived from fitting exponential curves are capable of being arranged so that they also increase as persistency increases, but in the form used by Gaines the measure of persistence is an inverse measure, the higher the arithmetic value the less the persistence.

Most of the authors mentioned above, and especially Gaines, have used their measure of the rate of decline in milk to study some of the general questions relating to milk production.

In addition to a consideration of the time relations the present paper will consider the question of the variation between lactations of a given cow and that of the herd. Pearl (21) has measured variability in milk production by means of efficiency percentages. His work presents these efficiency percentages according to the age of the cow and stage of lactation.

The variability of milk production from lactation to lactation has been studied by correlating first and later records of the same cows. Gowen (11, 15) found such coefficients of correlation between year's yield of one lactation and that of a later one to be between 0·5 and 0·8. Gavin (13), by a different and less easily followed technique, had in 1913 obtained a correlation coefficient of around 0·5 between first and later lactations. Sanders (23) used the variation of his "shape figure" to measure the variability of a cow, but his statistical method seems questionable. This work will, however, be discussed more fully later as we present the evidence of this study on the subject of individual variability.

MATERIAL.

The most of the scientific work on the present questions or on the related problems of heredity in cattle has been done with advanced registry records. The standards of the advanced registries are designed to select the best animals, and the best possible care is given them. This artificiality limits the usefulness of the advanced registry records. Even more crippling to the use of such records in studying lactation variation is the fact that the majority of cows are recorded only once.

It seems evident that to get at the true situation in regard to many of the general questions pertaining to milk production, different material is almost necessary. Such material is in existence in the records which form the basis of this study. These data overcome the objections existing in the advanced registry material, since they comprise the records of all the cows in a large pure-bred herd; the cows were well cared for but not artificially forced for production; and there are several records for many cows covering, in some cases, eight or ten lactation periods.

The material was assembled under the direction of Pearl (21) by Gowen (14). The herd consisted entirely of pure-bred Jersey cows, many of them imported directly from the island of Jersey. It was extremely well cared for and managed for many years by one man, in a favourable climate. The milk records used are all from normal, healthy cows, tuberculosis being eliminated according to the tuberculin test early in the history of the herd. There has been some abortion, but all records where an abortion occurred have been excluded.

The data used here are the production records of the herd (and this means all the cows of the herd—good, bad, and indifferent). The month-by-month milk yields are the basic data together with additional information incidental to such records. The original registers were very carefully kept, so that their accuracy is assured. In Gowen's work on this material (14), he found that 8 months was the longest period that would include the most of the milk records and still be long enough to give a real picture of the lactation. Therefore, 8-months' records are used in this study.

Certain facts about this herd may well be described at this point. The production records of the herd start with its beginning in 1897. There are 1741 complete 8-months' records. Some increase in the average 8-months' total yields of the herd has occurred during the period covered by these data. These averages fluctuate, yet the trend is distinctly upwards. There is a drop at 1905–6 due probably to importation of young island of Jersey stock (Gowen, personal communication), and the drop at 1909–10 may be due to the same cause. The standard deviations of these milk yields are quite variable, but in general they seem to be larger in the later years.

Arranging the lactations according to the month in which the calf was born and comparing the 8-months' total yields, this herd shows a distinct seasonal cycle. For the 1741 complete 8-months' records, the milk yield is lowest when the cow freshens in the summer, and it mounts steadily until the yield is highest if the cow calves in February.

The general opinion seems to be that cows that have their calves in the fall will produce the most milk, this opinion being held by Eckles (4) and several authors quoted by Turner (28). The latter says:

> "The fundamental reason for the variation in milk yield is probably one of nutrition. This in turn being affected to some extent by atmospheric temperature. Cows on test being well fed are probably less affected, either favourably or unfavourably, by pasture than those not being pushed for maximum production";

and Turner also says in the same paper:

> "From the standpoint of total production, cows calving in the fall and winter months equal or exceed the average production, while those calving during the summer months are generally below the average."

Turner's own data for the Jersey cattle, however, show a sharp rise in milk production for those cows that begin their lactations in July and August. Hammond and Sanders (18) show a slightly different situation from this herd. Their records are for Shorthorns of the milk-recording societies which are not selected for production as are the advanced registry records of Turner. The Shorthorns show a sudden drop in February, to make it the lowest point, but production stays low during the summer. Cows calving in the fall are higher and maintain a position above the annual mean. The rise begins before that of this herd, however. Sanders (23, 24, 25) for other groups of cattle from milk-recording societies shows similar trends.

It seems reasonable to suppose that the drop in the summer months which is observed in the English records and in this herd is more nearly the natural condition among dairy cattle, since the feeding conditions are probably not so artificially stabilised as they are for advanced registry cattle.

This material affords opportunity for the study of individual lactations and their relations to other factors. Because the variability of a cow from lactation to lactation is to occupy a prominent place in this study, the material was in the beginning arranged so as to do this efficiently. For this reason, cows were selected which had had at least six records, since fewer than this would be too small a sample with which to study individual variability effectively. There turned out to be ninety-nine such cows and they provided 679 lactations. Table 1 and Fig. 1 show average 8-months' milk yields for successive years for this sample. There are greater fluctuations than in the entire herd, but no greater than would be expected from the smaller numbers of records

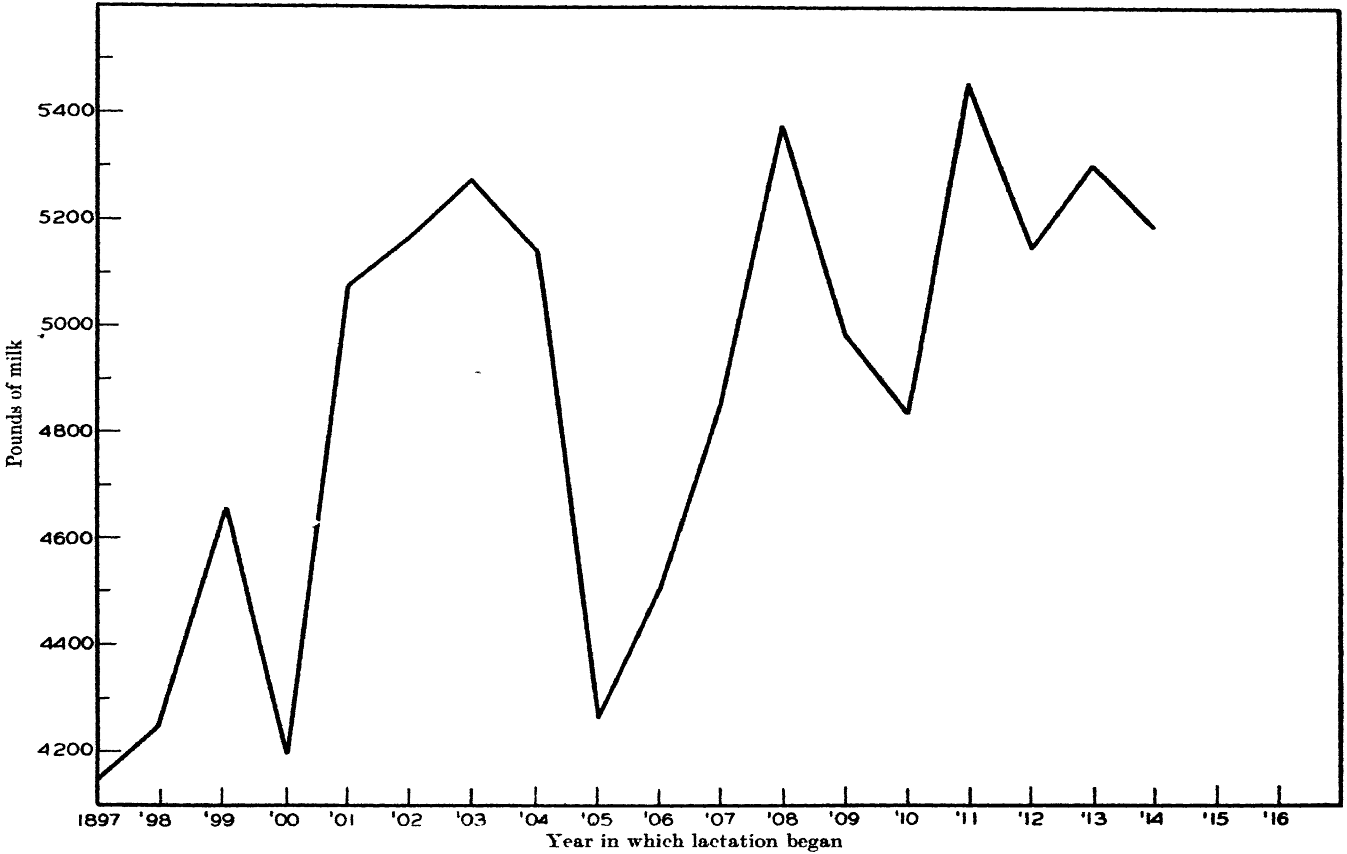

Fig. 1. Average 8-months' milk yields for successive years.

involved, the cows with six or more lactations being distributed in essentially the same way as the herd as a whole. The standard deviations of the selected 679 do not seem to increase in later years quite so much as they do for the entire herd, which might be expected, since the sample contains only cows that have had several lactations.

Table I. *Average 8-months' milk yields for successive years for the sample of 679 lactations. Cows having six or more lactations.*

Year in which record began	No. of records	Average yield lb.	Standard deviation lb.
1897	5	4150 ± 349	1158 ± 247
1898	9	4250 ± 205	913 ± 145
1899	15	4617 ± 157	903 ± 111
1900	18	4194 ± 109	685 ± 77
1901	15	5083 ± 241	1386 ± 171
1902	20	5175 ± 184	1217 ± 130
1903	31	5282 ± 163	1344 ± 115
1904	33	5144 ± 141	1205 ± 100
1905	45	4261 ± 113	1123 ± 80
1906	69	4504 ± 83	1027 ± 59
1907	63	4853 ± 93	1095 ± 66
1908	59	5377 ± 112	1281 ± 80
1909	58	4983 ± 96	1084 ± 68
1910	67	4832 ± 96	1170 ± 68
1911	52	5462 ± 107	1149 ± 76
1912	63	5147 ± 100	1182 ± 71
1913	49	5301 ± 132	1371 ± 93
1914	8	5188 ± 323	1356 ± 229

Table II. *Average 8-months' milk yields, by months, for cows having six or more lactations.*

Month lactation began	No. of records	Average yield lb.	Month lactation began	No. of records	Average yield lb.
January	72	5014 ± 96	July	46	4641 ± 99
February	63	5393 ± 90	August	62	4597 ± 91
March	48	5396 ± 114	September	57	4724 ± 111
April	47	5473 ± 128	October	66	4606 ± 102
May	51	5044 ± 99	November	55	4995 ± 110
June	39	5122 ± 124	December	73	5024 ± 102

Table II and Fig. 2 show that in respect to seasonal variation the same relation of the sample to the entire herd holds. That is, although the sample exaggerates the peaks and depressions of the whole herd, its seasonal distribution is not different from that of the herd. Examination of the above factors seems to show that these cows selected may be considered as representative of the entire herd.

DISCUSSION AND ANALYSIS.

A consideration of the form and mathematical constants of the time-change curves of individual lactations.

As was suggested above, month-by-month milk yields are approximately exponential in form, and considerable valuable information may be obtained by actually fitting such curves to milk-production data. Such a procedure gives a definite measure of the way milk yield varies with time during a single lactation. For this paper, exponential curves were fitted to every individual lactation record for all ninety-nine of the cows that had six or more lactations, giving a total of 679 curves[1].

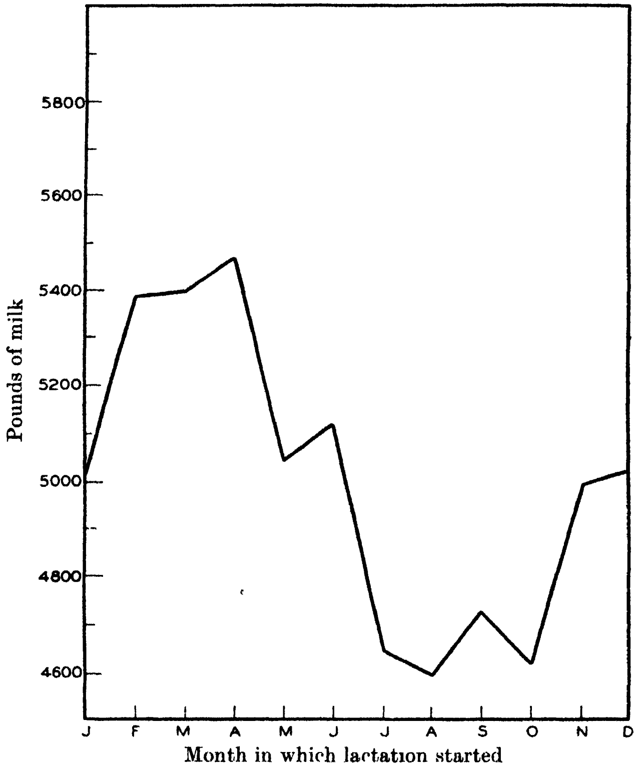

Fig. 2. Average 8-months' milk yields by months.

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[1] In the 679 records, there are seventeen that do not seem to follow the simple exponential curve with a decreasing yield. Of these, three are positive for the rate factor and fourteen are so extremely variable that no simple curve would fit them. Exclusion of these seventeen records does not change the averages of the curve constants, so they have been left in the sample. Two of these divergent records are from the same cow that had in all ten records.

The exponential curve of the form $y=ae^{bt}$ was fitted to the actual milk yields for the first 8 months of each lactation. In this form, y represents milk yield in pounds per month and t is time measured in units of one month. In this problem, the unknowns a and e^b, besides making it possible to put a smooth line through the observed points, have certain meanings. The a constant is the value of y at the 0-point on the time scale, that is, it is the initial yield. Since there is not an actual weighing of milk at the instant of calving, a is a theoretical measure. It is, however, a measure of initial milk yield and is expressed in pounds of milk.

The actual rate factor, as it stands in the equation, is e^b and has been used here to measure the monthly change of milk yield as lactation progresses. When e^b is more than 1, b is positive, and the curve climbs; when e^b is less than 1, b is negative, and the curve declines. This latter situation is the more common form of curve describing milk yield with time, although as has been said above, a few records show the reverse situation. When e^b is small numerically, the milk yield declines rapidly; the more nearly e^b approaches 1, the less the decline. It is, then, theoretically possible to have a cow start her lactation with a high initial yield (large a) and decline so rapidly (e^b small) that the total milk yield would be very little; or again, a cow might start rather low (small a) but maintain a constant level of production (e^b equal to 1) and so have a total yield that was larger than the first cow's. The constant e^b will then measure the swiftness of diminution from a, the initial yield, or it measures the persistency with which a cow produces milk, and the larger this constant is numerically, the more persistent is the cow.

Some measure is needed of how closely the smooth curve follows the actual data. Since the curves were fitted by making the sum of the squares of the deviations as small as possible, a root mean square error, or $\sigma_{y.x}$ is wanted. The $\sigma_{y.x}$ is defined as the square root of the sums of the squares of the deviations minimised in determining the curve, divided by the number of observations, n. In exponential equations the logs of the observed numbers are linear, and we have assumed that the logarithmic deviations about the smooth line are normally distributed. Therefore, a $\sigma_{y.x}$ has been calculated for each curve with the deviations regarded as the difference between logs of the observed and calculated y's. Since there are only eight observations for each curve, some correction should be made for the added variability due to the small sample. There are two constants necessary to determine the equations used and consequently the degrees of freedom are reduced by 2 and n becomes $n-2$ or 6. The $\sigma_{y.x}$ is, then, a measure of a cow's variability. If it is large, the milk yields will be very irregular, while a small $\sigma_{y.x}$ will mean that a cow's milk production declines very smoothly along an exponential equation. It constitutes an additional safety guard to the interpretations and by its use, we may be able to tell whether or not the variability of the material is masking or accounting for the given results.

Fitting exponential curves to the designated sample gives, then, three constants for every curve, or 679 a's, 679 e^b's, and 679 $\sigma_{y.x}$'s, representing theoretical initial yield, rate of decline, and variability about the fitted line, respectively. These are the foundations of this study, so it seems advisable to study these constants with some care. All three vary considerably from lactation to lactation but each forms a continuous frequency distribution.

The average value of the 679 a constants is 890 lb. ± 7 lb. That is, each cow, if she continued to give milk at the rate with which she began, would in a month produce on the average 890 pounds of milk. The average rate of decline is $0{\cdot}920 \pm 0{\cdot}001$. A given month's milk yield is, on the average, only 92 per cent. of the yield for the previous month. In this study, only records that had gone on for at least 8 months were used, so that the most rapid declines possible, such as would be the case if the cow stopped giving milk after 2 months, were excluded. But for a fairly normal length lactation, the persistency is 0·920. The average of the $\sigma_{y.x}$'s is $0{\cdot}064 \pm 0{\cdot}0004$. These statistical constants are given in Table III.

Table III. *Data for constants of* 679 *exponential curves fitted to individual* 8-*months' records of Jersey cows.*

	e^b	a	$\sigma_{y.x}$
Mean	$0{\cdot}920 \pm 0{\cdot}001$	$890{\cdot}06 \pm 7{\cdot}02$	$0{\cdot}064 \pm 0{\cdot}0004$
σ	$0{\cdot}040 \pm 0{\cdot}001$	$271{\cdot}23 \pm 4{\cdot}96$	$0{\cdot}017 \pm 0{\cdot}0003$
β_1	$0{\cdot}19 \pm 0{\cdot}10$	$0{\cdot}23 \pm 0{\cdot}05$	$1{\cdot}42 \pm 0{\cdot}33$
β_2	$3{\cdot}85 \pm 0{\cdot}60$	$3{\cdot}03 \pm 0{\cdot}15$	$5{\cdot}34 \pm 1{\cdot}00$

$$r_{e^b a} = -0{\cdot}585 \pm 0{\cdot}017$$

$$r_{e^b \sigma_{y.x}} = -0{\cdot}225 \pm 0{\cdot}025$$

$$r_{a\sigma_{y\,x}} = +0{\cdot}088 \pm 0{\cdot}026$$

All three distributions are skew, as is shown by the β's. For e^b, $\beta_1 = 0{\cdot}19$ and $\beta_2 = 3{\cdot}85$; for a, $\beta_1 = 0{\cdot}23$ and $\beta_2 = 3{\cdot}03$; for $\sigma_{y.x}$, $\beta_1 = 1{\cdot}24$ and $\beta_2 = 5{\cdot}34$. In a normal curve $\beta_1 = 0$ and $\beta_2 = 3$, so that these constants probably are not normally distributed but they do not seem so far from normal as to prohibit the use of correlation coefficients.

The correlation between e^b and a is $r = -0{\cdot}585 \pm 0{\cdot}017$. That is, high initial yield tends to be associated with a rapid decline of milk, or conversely, the persistent milker starts at a relatively low level which is maintained more or less closely. The correlation of the rate factor and the standard deviation about the fitted line is $r = -0{\cdot}225 \pm 0{\cdot}025$, a low but statistically significant correlation. The more persistent milkers tend to have less variability, but this relation is not very accurately determined with such a low correlation coefficient. Initial milk yield shows practically no correlation with the standard deviation, $r = 0{\cdot}088 \pm 0{\cdot}026$. There is practically no tendency for the cows with a high initial yield to be more variable in their deviations from the smooth curve.

Gaines (9) has arrived at somewhat similar results using the same type of curve. He has fitted exponential curves to averages of monthly yields and also to individual records for certain advanced registry animals of both Holstein-Friesian and Guernsey breeds. Gaines' technique differs but little from that used in this study. His a constant is not directly comparable with that of this study since all his records are converted into an index number, fat-corrected milk. He also uses in all his work b rather than e^b as a measure of rate. It should be remembered in this connection that Gaines' measure of persistency is an inverse rate, that is, the higher the numerical value, the less the persistency. This rate may, however, be converted into e^b, and doing this for his average b gives an average rate which is equivalent to an $e^b = 0{\cdot}956 \pm 0{\cdot}001$ for Guernsey cows. The figure for this herd as given above was $0{\cdot}920 \pm 0{\cdot}001$, indicating that the Guernsey advanced registry animals are more persistent in their milk yields. Gaines (9) says that the Holstein-Friesians are more persistent than the Guernseys, which might be expected since the Holsteins give generally more milk in a lactation and there must be some relation between the two things. In some later work with Brown Swiss cattle, Gaines and Brock (10) get a persistency value of 97 per cent. Ellinger (5) finds Red Danish cattle to be more persistent than Jerseys. He uses a different technique, not a curve-fitting method, so that the results cannot be compared arithmetically. Sturtevant (27) had earlier reported a 9 per cent. decrease for a private herd of several different breeds. Turner (29) gets a persistency value of 94·8 per cent. Some of these figures are from advanced registry records and some from private herds with different environmental conditions so that these comparisons cannot be taken too literally, but it does seem fair to say that there are differences between breeds in persistency of producing milk.

Eckles (4) explains the negative relations between high initial yield and persistency as being due to lack of adequate food. He says that the food requirements for a cow giving milk are enormous. In fact, the cow usually has to draw on her own bodily reserves to maintain the milk production. On p. 292, Eckles says that a cow producing the very ordinary amount of 30 lb. of milk a day is producing 3·75 lb. of dry matter in her milk which is more than twice as much as a steer would add to his body weight if he were making a gain of 2 lb. of actual weight, since this would represent only 1·5 lb. of actual dry matter. Gaines (6, 9) gets a correlation coefficient between initial yield and persistency of 0·535 ± 0·012 for Guernseys and 0·407 ± 0·019 for Holstein-Friesians. Although these coefficients are expressed as positive values, they have a negative relation since the persistency factor as Gaines uses it is an inverse rate. Gaines' coefficients are slightly smaller than that found here, but express the same relation, that persistency of a cow decreases as initial yield increases. Sanders (26) in 1930 also found a negative relation between maximum yield and persistency, although he arrived at this result by a different mathematical process.

The relation of the constants of the time-change curves of individual lactations to the year in which the lactations began.

If a herd of cows is to be profitable and an efficient machine, one would expect some selection toward higher yield to be taking place. Such selection has been taking place in this herd. By studying the relation of the time-change curve constants, it is possible to see how the selection was brought about. The averages and their probable errors of the curve constants for each year are given in Table IV. The relations of the e^b constant with the year when lactation started seem to disprove

Table IV. *Curve constants for the year when lactation began.*

Year	No. of records	Average e^b	Average a	Average $\sigma_{y.x}$
1897	5	0·921 ± 0·007	710 ± 56	0·045 ± 0·003
1898	9	0·941 ± 0·007	683 ± 41	0·053 ± 0·006
1899	15	0·923 ± 0·005	803 ± 32	0·062 ± 0·002
1900	18	0·929 ± 0·007	717 ± 29	0·065 ± 0·002
1901	15	0·904 ± 0·010	963 ± 50	0·068 ± 0·003
1902	20	0·911 ± 0·007	970 ± 53	0·065 ± 0·002
1903	31	0·921 ± 0·005	921 ± 29	0·056 ± 0·003
1904	33	0·923 ± 0·003	905 ± 26	0·060 ± 0·002
1905	45	0·937 ± 0·004	783 ± 24	0·061 ± 0·002
1906	69	0·928 ± 0·003	773 ± 17	0·066 ± 0·001
1907	63	0·934 ± 0·002	804 ± 17	0·064 ± 0·001
1908	59	0·916 ± 0·003	982 ± 25	0·065 ± 0·001
1909	58	0·921 ± 0·003	876 ± 23	0·064 ± 0·002
1910	67	0·909 ± 0·004	907 ± 23	0·063 ± 0·001
1911	52	0·919 ± 0·003	979 ± 23	0·063 ± 0·001
1912	63	0·908 ± 0·004	979 ± 26	0·068 ± 0·002
1913	49	0·910 ± 0·004	989 ± 29	0·063 ± 0·001
1914	8	0·905 ± 0·009	963 ± 51	0·070 ± 0·005

that the selection was for persistent milkers. The initial yield varies markedly with time, but the general trend probably means some retention of cows that start the lactations with a relatively large amount of milk. Average variability ($\sigma_{y.x}$) also fluctuates somewhat with time, but there seems to be some increase in variability during the later years covered by these records, indicating that the selection taking place within the herd served to increase the average variability.

The actual years covered here have no significance except in this herd's history but the author can find no data in the literature where the differential effect of the two curve constants on the increasing yields of the same herd has been studied. In this herd where some selection is taking place, it is apparently being attained by selection toward high initial yield rather than consistent performance.

The relation of the constants of the time-change curves for individual lactations to the month in which the lactations began.

Before cows were so artificially fed and cared for, the season of year when the calf was born was more important than it is to-day, but there are distinct seasonal trends even in modern herds. Table II illustrates this cycle for the total yields of this sample of one herd. The means and their probable errors of the curve constants for each calendar month of calving are shown in Table V, while Figs. 3 and 4 show these relations graphically. The cow whose milk yield declines most rapidly, calves in April, while October, November, December, and January are likely to produce more persistent milkers. The difference between the average e^b constant for April and the average for December is very significant statistically, being more than 13 times the probable error of the difference.

Table V. *Curve constants for cows calving in various months of the year.*

Month when lactation began	No. of records	e^b Mean	a Mean	$\sigma_{y.x}$ Mean
January	72	0·941 ±0·003	821 ±21	0·061 ±0·001
February	63	0·911 ±0·003	998 ±22	0·068 ±0·001
March	48	0·902 ±0·003	1025 ±25	0·073 ±0·002
April	47	0·890 ±0·003	1093 ±27	0·060 ±0·001
May	51	0·897 ±0·004	997 ±23	0·067 ±0·001
June	39	0·900 ±0·005	983 ±27	0·064 ±0·001
July	46	0·907 ±0·004	878 ±25	0·063 ±0·002
August	62	0·912 ±0·004	847 ±24	0·061 ±0·002
September	57	0·924 ±0·003	818 ±23	0·064 ±0·002
October	66	0·938 ±0·003	755 ±17	0·063 ±0·001
November	55	0·942 ±0·003	812 ±20	0·062 ±0·002
December	73	0·944 ±0·003	803 ±19	0·060 ±0·001

Almost the exact reverse occurs with initial yield and season. April calvers have a higher average *a*. Since the cows with a high *a* constant calve at the same time as do those with a high total yield, it suggests that initial yield is more important than the rate factor in determining high total yield.

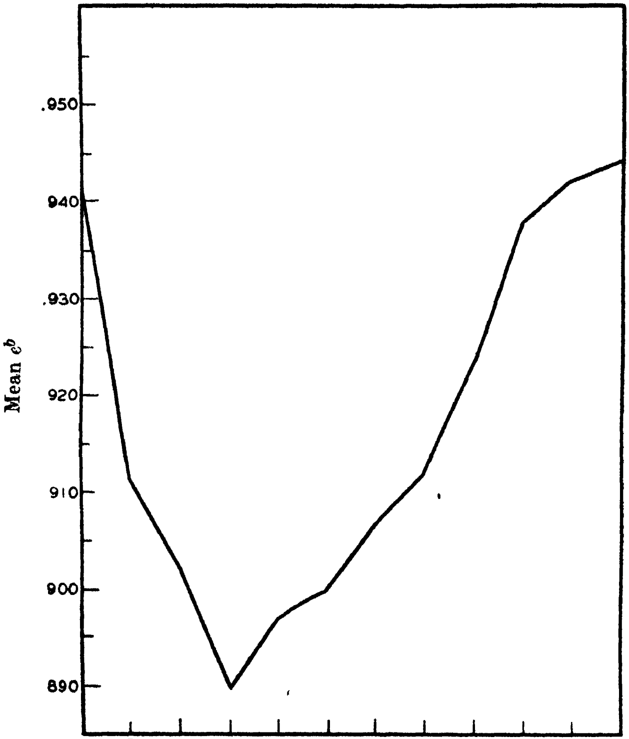

Fig. 3. Persistency rate for cows calving in various months of the year.

The measure of variability of the observations about the fitted line shows no seasonal cycle, the fluctuations being so great that no trend can be seen. This lack of relation indicates that the previously shown relations of season with initial yield and with persistency are not invalidated by greater variability of the curves which began at particular times of the year.

The coefficient of correlation between e^b and *a* for the whole sample of 679 records is $-0{\cdot}585 \pm 0{\cdot}017$ (see Table III). Some of this correlation may be due to the fact that almost throughout the year the e^b is high when the *a* is low and *vice versa*. If we, in effect, hold season constant, this correlation persists at almost the original value. This was accomplished by selecting a group of cows calving in February, March, April, and May (when e^b is low and *a* high) and another group calving in September, October, November, and December (when e^b is high and *a* low), and correlating the e^b and *a* for the two groups. The coefficients obtained are $-0{\cdot}514 \pm 0{\cdot}034$ for the spring calvers and $-0{\cdot}408 \pm 0{\cdot}035$ for the winter calvers. These coefficients are so much like the original correlation that the seasonal change of e^b and *a* does not seem to be the major source of the correlation between the variables.

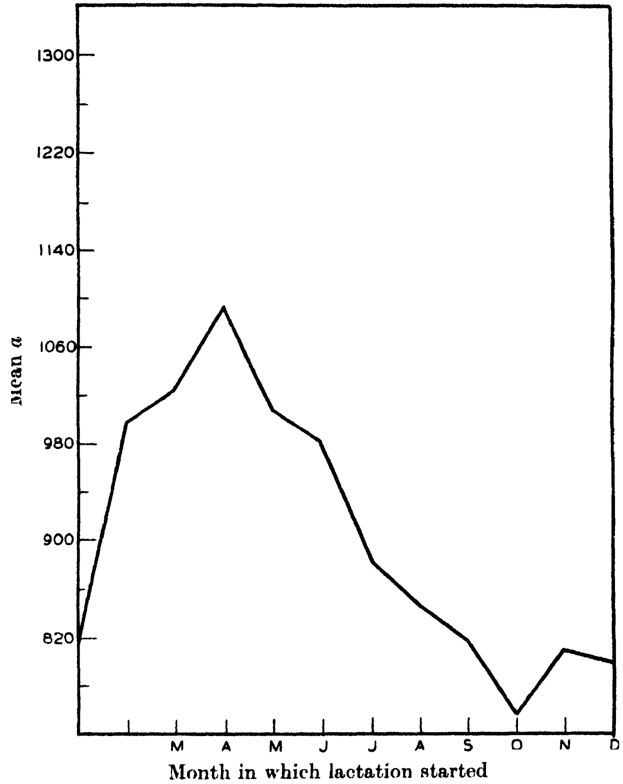

Fig. 4. Theoretical initial yield for cows calving in various months of the year.

Gaines (9) with Guernsey cows gets no such pronounced reversal of initial yield and rate with season although he says: "...apparently cows calving in the summer months hold up somewhat better than cows calving in the winter months when initial yield is allowed for." His constants have been corrected for age, fat content, and other factors, so that the figures cannot be compared directly with these results, but they seem to be opposite in respect to the persistency factor.

The relation of the constants of the time-change curves for individual lactations to the length of time during a given lactation that a cow was pregnant.

Since the fact that a cow is giving milk does not prevent her from breeding, the lactating animal may be pregnant for various lengths of time during the period studied by these milk records. In this study curves are fitted to yields for the first 8 months only, so that they cannot get nearer than 1 month to the time of parturition, as the gestation period is approximately 9 months.

The information necessary to determine the time of breeding is available for 562 records of the group to which curves were fitted. These records were classified according to the length of time the calf was carried. All these records where this time was less than 5 months were considered together, since there is generally believed to be no effect before this time. This gives five classes for these data—less than 5 months, 5 months, 6, 7, and 8 months. The average monthly yields for these five classes are shown in Fig. 5, plotted on arithlog paper so that the trends may be compared directly. This shows that there is no significant change in the shape of the curve, nor significant drop at the end, if the variability, which unquestionably exists, is considered.

As there is no disturbance of these milk yields associated with the advance of pregnancy, the latter may be studied in relation to the time-change curve constants. The distributions of the months the calf was carried is too far from normal to use a correlation coefficient to measure the relation of the duration of pregnancy to any other variable. The averages from month to month will, however, indicate any tendency for the two factors to vary together. These means and their probable errors are shown in Table VI. Neither the mean e^b, the mean a, or the mean $\sigma_{y.x}$ shows any trend with advance of pregnancy. The variability of the points about the fitted line not only shows no trend, but with one exception all the means are within their probable error limits, like the mean of the entire sample.

Brody, Ragsdale and Turner (2, 22), Turner (29), and Gaines and Davidson (11, 12) found that the shape of the lactation curve changes if the cow has carried a calf during a major portion of the lactation. Brody and his co-workers separate from the farrow group cows that are bred

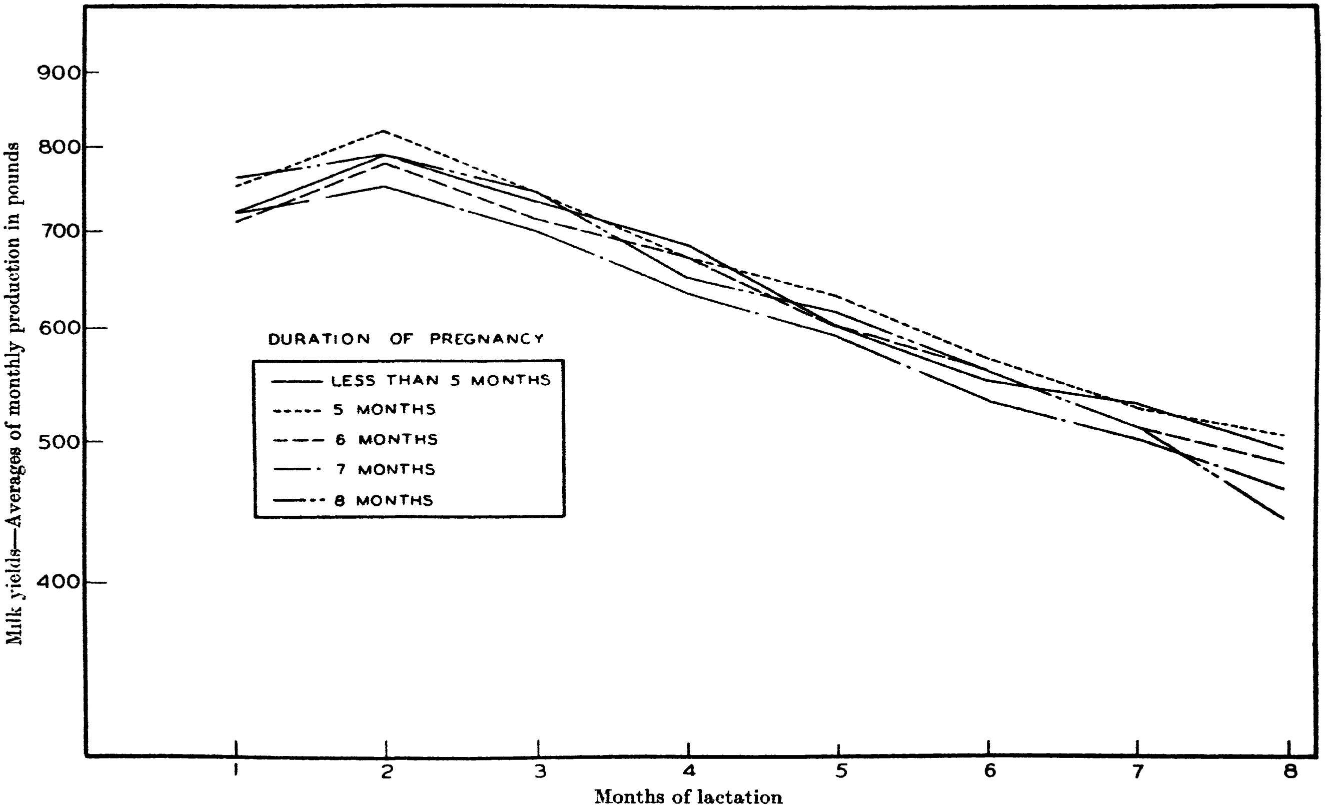

Fig. 5. Average milk yield for cows carrying calves varying lengths of time.

during the third or fourth months of lactation. The milk yield of the pregnant group drops below that of the farrow group all along, with a marked bend between the tenth and twelfth months of lactation. Since these cows were bred in the third or fourth month of the lactation, this drop in production must be immediately before parturition. Gaines and Davidson (11) consider together all the farrow cows and those bred 5½ months or more after calving because of the similarity of the lactation curves. If the cows were bred earlier than 5½ months from calving, there was a decided downward bend to the lactation curve.

Table VI. *Curve constants for different durations of pregnancy within a lactation.*

Months carried calf	e^b Mean	a Mean	$\sigma_{y.x}$ Mean
0	0·928 ±0·005	853 ±33	0·066 ±0·002
1	0·929 ±0·006	919 ±42	0·064 ±0·002
2	0·920 ±0·005	866 ±25	0·065 ±0·003
3	0·937 ±0·005	911 ±32	0·063 ±0·002
4	0·923 ±0·004	920 ±26	0·065 ±0·001
5	0·931 ±0·003	908 ±21	0·064 ±0·001
6	0·925 ±0·003	900 ±19	0·061 ±0·001
7	0·925 ±0·002	863 ±14	0·063 ±0·001
8	0·914 ±0·003	950 ±22	0·066 ±0·001

On the other hand, Gowen (16) with Guernsey cows shows that there is little effect of the foetus on the energy the cow is able to convert into milk. His results are reached from an entirely different point of view than that of the authors quoted above. From the regression equations of milk yields at different ages, he finds the reduction in milk due to the foetus to be from 400 to 600 lb. of milk. On this basis, the nutrient requirement of the foetus is about 3–5 per cent. of the milk produced. Consequently, the foetus actually uses relatively little of the total energy the cow is able to produce. Eckles (3) in 1916 had found experimentally that a "...cow can produce a fetus on the same ration that will maintain her when dry and farrow," and (4) concludes that it is more of a drain on a cow to maintain milk production.

Thus it will be seen that this study, which does not consider the lactation during the final month of pregnancy, agrees with the views of Gowen and Eckles in regarding the drain of pregnancy as slight.

These observations as to whether gestation does or does not seriously affect milk yield are concerned with the effect on total yields, not measure of persistency or initial yield. Sanders (23) states that persistency rises as the service period lengthens, or in other words as the duration of pregnancy during a lactation increases. In the papers referred to in the paragraphs above, Gaines uses exponential lactation curves and compares the slopes and declines before parturition, but there is no explicit statement as to how the curve constants vary with increasing gestation. In another paper Gaines (7) studied this question using Guernsey Advanced Registry records where the tendency is to delay breeding with the idea of increasing the year's milk, and which are at least approximately normally distributed as to length of service period. He found there a very low although statistically significant correlation between initial yield and service period, $r = 0{\cdot}039 \pm 0{\cdot}010$. So that these Jerseys with their somewhat different treatment seem to behave like the Guernseys as showing no relation of the curve constants to length of pregnancy.

The relation of the constants of the time-change curves for individual lactations to the age of the cow at the beginning of the lactation.

The age distribution of the 679 records is artificially loaded at the higher ages, since no cows are included in the group that are not old enough to have had six calves, and there are a good many more in the herd with fewer than six records. For this reason, a correlation coefficient between the curve constants and age is meaningless, but the relation may be studied by considering the means of the constants for the successive age groups. The means of the constants for the different age groups are given in Table VII.

Table VII. *Curve constants for successive ages.*

Age	No. of records	e^b Mean	a Mean	$\sigma_{y.x}$ Mean
1·5–2·4	72	0·942 ± 0·002	654 ± 11	0·057 ± 0·001
2·5–	63	0·922 ± 0·003	787 ± 18	0·064 ± 0·001
3·5–	67	0·924 ± 0·003	880 ± 18	0·063 ± 0·001
4·5–	65	0·919 ± 0·003	947 ± 24	0·063 ± 0·001
5·5–	83	0·922 ± 0·003	933 ± 19	0·061 ± 0·001
6·5–	82	0·911 ± 0·003	987 ± 21	0·070 ± 0·002
7·5–	87	0·914 ± 0·003	967 ± 19	0·065 ± 0·001
8·5–	68	0·917 ± 0·004	907 ± 25	0·067 ± 0·001
9·5–	38	0·910 ± 0·004	961 ± 29	0·058 ± 0·001
10·5–	25	0·920 ± 0·005	850 ± 37	0·066 ± 0·002
11·5–	14	0·898 ± 0·006	914 ± 46	0·063 ± 0·003
12·5–13·4	12	0·928 ± 0·010	817 ± 55	0·069 ± 0·003

The mean e^b's for successive age groups, while they are very variable, do show a tendency to be higher in the lower age groups. That is, the more persistent milkers are the younger cows. By averaging the a's for successive age groups, a tendency is seen for the older cows to start their lactation high. The older cows are also more variable as measured by the average $\sigma_{y.x}$.

If the records are divided into three broad age groups and the correlation coefficients between e^b and a calculated, we find that this correlation increases with age. Table VIII shows these values. The first group, including those animals from 1·5 to 3·5 years, has a correlation of $-0{\cdot}453 \pm 0{\cdot}046$. The cows aged from 4·0 to 7·5 years have a coefficient of $-0{\cdot}569 \pm 0{\cdot}027$, while the older cows from 8·0 to 15·0 years have a coefficient of $-0{\cdot}607 \pm 0{\cdot}027$. This shows, then, that the correlation between initial yield and persistency is higher at the time in the cow's life when the initial yield is high.

Table VIII. *Correlation of persistency and initial yield for three age groups.*

Age in years	n	Correlation coefficient
1·5– 3·5	135	$-0{\cdot}453 \pm 0{\cdot}046$
4·0– 7·5	297	$-0{\cdot}569 \pm 0{\cdot}027$
8·0–15·0	247	$-0{\cdot}607 \pm 0{\cdot}027$

Sanders (23) found no change in the "shape figure" which he uses as a measure of persistency, after the second lactation. Gaines and Davidson (11) and Gaines (9) found that older cows are less persistent than younger ones. The association is more marked with Guernseys than with Holsteins. Gaines (9) also finds that the older cows have a higher initial yield than the younger ones and that here the relation is closer with the Holsteins. In some more recent work, Gaines and Brock (10) found with Brown Swiss cattle that persistency was not affected by age.

Turner (30) also observed essentially the same relation with age that has been shown in this study. His measure of persistency was different, being an average percentage of 1 month's yield to that of the preceding month, but he says that the maximum month's secretion increases rapidly with age, and while persistency declines up to the age of maturity, the maximum month increases more rapidly, so that the end result is the increased production with advancing age.

The relation of the constants of the individual time-change curves to the length of the time that the cow was dry before the given lactation.

For 583 of the records to which curves were fitted, it is possible to determine how long the cow was dry before the record began. This resting period lasted from 0 to 109 weeks, but in most of the cases was under 30 weeks. A correlation coefficient would be meaningless because the distribution of frequency is so very skew. Means for the curve constants can be compared from week to week of the dry period, however, as is done in Table IX. Only the first 16 weeks have large enough frequencies to make statistical constants of much value.

Table IX. *Curve constants for varying lengths of dry period.*

Weeks dry before given lactation	No. of cords	e^b Mean	a Mean	$\sigma_{y.x}$ Mean
0	10	0·926 ± 0·008	750 ± 45	0·068 ± 0·005
1	5	0·931 ± 0·005	670 ± 23	0·067 ± 0·001
2	16	0·939 ± 0·005	694 ± 25	0·066 ± 0·003
3	18	0·926 ± 0·007	761 ± 26	0·058 ± 0·002
4	22	0·930 ± 0·008	777 ± 29	0·062 ± 0·002
5	27	0·925 ± 0·005	798 ± 30	0·064 ± 0·002
6	48	0·930 ± 0·003	852 ± 24	0·062 ± 0·002
7	49	0·928 ± 0·004	897 ± 22	0·060 ± 0·001
8	60	0·922 ± 0·004	952 ± 26	0·067 ± 0·001
9	62	0·912 ± 0·003	989 ± 24	0·068 ± 0·002
10	60	0·908 ± 0·003	1007 ± 23	0·074 ± 0·001
11	44	0·910 ± 0·004	986 ± 28	0·066 ± 0·002
12	30	0·912 ± 0·004	943 ± 29	0·061 ± 0·002
13	23	0·904 ± 0·007	924 ± 51	0·068 ± 0·003
14	18	0·920 ± 0·004	944 ± 33	0·061 ± 0·002
15	16	0·898 ± 0·006	1100 ± 48	0·068 ± 0·003
16	15	0·919 ± 0·006	943 ± 53	0·073 ± 0·004

The rate factor, e^b, shows a decided decline as the dry period increases up to 16 weeks. After that time the observations scatter very widely due to small numbers. The more persistent milkers do not have a very long rest period before the lactation, or conversely, the cows that were dry for more than 8 weeks tend to fall off in their milk yield more rapidly than the average of the whole sample. This result may represent a real biological condition or it may be artificial and due to the practice of breeding earlier the cows that had maintained a relatively high milk yield. The latter seems to be the case here when the average persistency of a cow throughout her several lactations is considered. Correlating deviations from the mean e^b for a cow with weeks dry gives a coefficient of $-0{\cdot}094 \pm 0{\cdot}028$, indicating that a cow's persistence relative to her own average does not decrease markedly with increasing dry period. There is no indication that some cows are always bred early in their lactations and others are always given a prolonged rest.

The initial yield, a constant, shows on the other hand, that these averages increase with the resting period. The cow that has rested 7 weeks or longer starts her next lactation with an initial yield that is above the average of the 679 records.

The mean $\sigma_{y.x}$'s fluctuate very widely, but there is some tendency for increased variability to be associated with longer dry periods. Cows that are very variable in their time relation to milk yield tend to have longer than average dry periods, but these $\sigma_{y.x}$'s are very irregular.

Gaines (9) does not analyse this effect of dry period on the curve constants. He merely states that practical experience has shown that it is desirable to allow 1–2 months dry period before calving. Sanders (23) in 1923 found no significant relation between dry period and the "shape figure," his measure of persistency. In a paper published in 1930, Sanders (26) finds that a resting period of 40 days is necessary for full development of maximum yield, but that any further rest period is reflected only in increased persistency.

So that the common practice of allowing a dry period of 6 weeks to 2 months, which Eckles (4) says is desirable, is undoubtedly justified by the increased initial yield which will result.

The relation of the constants of the individual time-change curves to the total number of weeks that a cow gives milk.

These cows were not being tested for entrance into an advanced registry which requires a year test, so that the duration of lactation varies exceedingly. While the curves were fitted to the first 8-months' yields only, the actual length of the lactation is known in all cases. This distribution is disturbed because of the fact that all the cows used had given milk for at least 8 months and, consequently, none of the exceedingly short records are included. This artificial cut at the low end of the range of lactation length results in distributions that are too skew to allow the use of correlation coefficients. The longest records run for over 2 years, but there are so few of the extremely long records that the last 40 weeks have been grouped together. Comparison of the averages of the curve constants for consecutive 10-week periods, as is done in Table X, shows certain definite trends.

The rate factor, e^b, increases as the lactation lengthens which would to some extent have to be the case, for if the milk yield declined very rapidly it could hardly go on for 2 years. The average a constant decreases as the lactation becomes longer. The longer records tend to start low and maintain nearly the same level throughout. As might be expected from this, the long, slowly declining milkers tend to have less variability, as was also suggested in the correlation of e^b and $\sigma_{y.x}$. All these trends with length of lactation are merely trends, with considerable variability—not extremely marked relation, but statistically significant.

The variabilities of each constant as measured by the standard deviations of the distributions of the curve constants show some interesting things. They all decrease for about a year and then increase. So that aside from the difference due to smaller numbers of individual records, there is an increase in variability after what might be called a normal length of lactation. This might be interpreted as indicating the existence of an optimum length of lactation of such a character that deviations from this optimum result in more variable performance.

Table X. *Curve constants for different lengths of lactation.*

		c		a		$\sigma_{y.x}$	
Weeks in milk	No. of records	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
30–39	104	0·893 ± 0·003	0·041 ± 0·002	975 ± 20	300 ± 14	0·070 ± 0·001	0·020 ± 0·001
40	328	0·922 ± 0·001	0·038 ± 0·001	889 ± 10	269 ± 7	0·063 ± 0·001	0·017 ± 0·000
50	154	0·930 ± 0·002	0·032 ± 0·001	864 ± 8	241 ± 9	0·062 ± 0·001	0·014 ± 0·001
60	47	0·927 ± 0·004	0·039 ± 0·003	859 ± 22	219 ± 15	0·062 ± 0·002	0·017 ± 0·001
70	20	0·902 ± 0·007	0·047 ± 0·005	945 ± 48	320 ± 34	0·070 ± 0·003	0·022 ± 0·002
80	14	0·956 ± 0·011	0·063 ± 0·008	707 ± 59	329 ± 42	0·061 ± 0·002	0·012 ± 0·002
90–129	12	0·929 ± 0·005	0·047 ± 0·003	824 ± 31	312 ± 22	0·064 ± 0·002	0·018 ± 0·001

There has been very little analysis of the actual number of weeks a cow gives milk, in terms of curve constants. Ellinger (5) finds no relation of either the maximum daily yield (comparable to our a constant) or persistency to length of lactation, while in this study there seems to be a tendency for the persistency factor to decrease with length of record and for the a to increase.

The relation of the constants of the time-change curves for individual lactations to 8-months' total milk yield.

The suggestion made above, that the e^b constant is less directly connected with the total yield than is the a constant, may be verified directly by correlation of the constants with total yield. The curve constant which measures a cow's persistency of milking shows no significant correlation with the 8-months' total yield ($r = -0{\cdot}050 \pm 0{\cdot}026$), indicating that there is no marked tendency for the more than average persistent cow to give more or less than average quantity of milk.

Since the total yield must be due to the combination of initial yield and rate of production, and since the rate shows no correlation with total yield, the initial yield should be highly correlated with milk yield. This relationship is indicated by the coefficient $r = 0{\cdot}847 \pm 0{\cdot}088$. Such a correlation means that cows that are going to give more than average amount of milk in 8 months, start their yield high. This correlation between high initial yield and total production suggests that this a constant would be very valuable in estimating the worth of a cow early in her lactation period. But this a constant is a theoretical measure of milk yield and the best approximation we have to it in actual pounds of milk is the first month's yield. The correlation between the a constant and the first month's yield is 0·633 ± 0·016. The correlation between the first month's yield and 8-months' total yield is 0·691 ± 0·014. So that as an approximation to the a constant which has been shown to be a valuable index of a cow's worth, the use of the first month's actual yield is justified. The fact that the initial yield is important in estimating a cow's milk yield may be considered to support the use of short tests for advanced registry cattle instead of the customary year tests, if the short test is made at the beginning of the lactation.

The variabilities of the monthly yields about the fitted line show no association with 8-months' yields ($r = -0{\cdot}017 \pm 0{\cdot}026$). The correlation again is negative but less than its probable error, so that it is not statistically significant. Variability, therefore, is about the same for high or low producers.

The correlation coefficients referred to in this section are summarised below:

Factors correlated.	
8-months' total yield with e^b	−0·050 ± 0·026
8-months' total yield with a	+0·847 ± 0·008
8-months' total yield with $\sigma_{y.x}$	−0·017 ± 0·026
8-months' total yield with first month's yield	+0·691 ± 0·014
First month's yield with a constant	+0·633 ± 0·016

While nearly all of the people working on milk production in the early 1920's considered the importance of persistency in estimating milk production, the results are somewhat varied and confusing. Sanders (23) compared the "shape figure" (his estimation of persistency) of cows grouped as to total milk yield into low, moderate, and high yielders and found that the "shape figure" increased with production. That is, more persistent milkers gave more milk. Ellinger (5) in the same year found no relation between maintenance ability and length of lactation. This indirectly measures the relation with total yield since the latter must depend in no small measure on length of lactation. Gaines (9) a few years later did correlate directly both the persistency factor and the initial yield with the year's production. He found the same relations that were found in this study, namely that as compared with initial yield, persistency is much less important in determining production.

The difference in the constants of the time-change curves for the herd as a whole and from lactation to lactation of one cow.

The standard deviations of e^b and a, as given in Table III, show the variability of these constants for the herd, at least as far as the sample of 679 records measure it. These standard deviations are $\sigma_{e^b}=0{\cdot}040$ and $\sigma_a=271{\cdot}23$. These figures will be used in later sections and will be referred to as $\sigma_{\text{of the herd}}$.

Some cows contributed six, some seven, some eight, some nine, some ten records to the total of 679. Consequently, the records were divided according to the number of lactations a cow had provided[1]. These sub-distributions give the following standard deviations:

	$n-1$	No. of cows	σ_{eb}	σ_a
6 lactations	275	46	0·039	260·53
7 lactations	216	31	0·039	264·61
8 lactations	111	14	0·041	320·72
9 lactations	53	6	0·041	224·07

Since there are only two cows with ten lactations each, they have not been used. These standard deviations are designated *standard deviation of the herd* with the subscript 6, 7, 8, or 9 to distinguish the subgroup.

Sanders (23) has a standard deviation of the "shape figures" for the group of cows but it is so different mathematically from the e^b constant that there seems little point in giving Sanders' figures.

The e^b's and a's for the several lactations of each individual cow were made into separate distributions and standard deviations of these distributions calculated. These standard deviations of the separate cows were then grouped according to the number of lactations the cow had, to compare with the subgroups of the herd as given above. The average standard deviation for each group of individual cows was calculated by determining the square root of the average variance, variance being defined as the square of the standard deviation. The procedure gives the following values:

	$n-1$	Average σ_{eb} between lactations	Average σ_a between lactations
6 lactations	45	0·038	217·73
7 lactations	30	0·035	204·91
8 lactations	13	0·040	252·42
9 lactations	5	0·041	246·58

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[1] Throughout the subsequent discussion, the standard deviations were calculated by the small sample method. This becomes essential later when the number of lactations for a cow becomes the size of the sample and the same procedure was adopted from this point on. So that $n-1$ is used in all cases to divide the squared deviations from the mean.

The total variability of the 679 records, or similarly of the 276 records of cows having six lactations each, must consist of variability between lactations of single cows plus variability between cows. The total herd variability and the variability from lactation to lactation are the standard deviations just considered. Adjusting for the loss of degrees of freedom due to sampling, the following relationship, which holds for the universe from which the sample is drawn, may be used:

$$\sigma_{\text{between cows}} = \sqrt{\sigma_{\text{herd}}{}^2 - \sigma_{\text{between lactations}}{}^2}.$$

The results for the three cases, cows with six, seven, and eight lactations each, are shown below. The case of nine lactations is dropped at this point since the sampling variability is so great as to mask all other effects.

	Average σ_{lb} between cows	Average σ_a between cows
6 lactations	0·010	143·07
7 lactations	0·017	167·42
8 lactations	0·012	197·85

Comparing these results with those given above for between lactations, we find the somewhat unexpected result that these cows vary more between the lactations of one cow than between cows in respect to the time-change curve constants.

Sanders (23) made a similar attack on the problem of differences between cows and between lactations of the same cow. He subtracted standard deviations of individual lactations from the standard deviation of the herd, but he did not square the deviations before he subtracted them, so that arithmetically his results are wrong. But if his figures are corrected for this error, his conclusion still holds, namely, that the cows differ more from each other than do the records of a given cow. In comparison with this result, this material seems to be more variable from lactation to lactation than might be expected. This probably means that this particular group of cows is relatively homogeneous, genetically.

The variability of the time-change curve constants for a cow of constant age and the average of a group of cows of the same age.

These estimations of variability take no account of age. It would seem probable that the effect of age would be felt more in the case of the individual cow, since there must be practically a year's difference in age between lactations. What is really wanted is a comparison of a cow, when variation due to age is eliminated, and the average of a group of cows of the same age. Consequently, the next step is to determine how much variability is due to age and what variability exists between cows of the same age.

The three groups—six, seven, and eight lactations—were divided by age classes. This gives distributions of e^b and a for each year of age. In only two of the age groups does the same cow appear twice, so that a given age group represents records from different cows of the same age, and the standard deviation of each distribution will measure the variability between cows of the same age. But an average standard deviation for these age groups introduces the effect of the same cow repeated at different ages. Consequently, this latter is of the order of the standard deviation of the herd, eliminating the variation due to age. The same type of analysis used in the section above gives the variability due to age as the difference between the total herd variation and the herd variation, eliminating the variation due to age.

In getting the average standard deviation for the age groups, account was taken of the size of sample in each age group and of the degrees of freedom lost through determining the standard deviation for each group. That is, in the case of the six lactations group, twelve age groups were averaged and in determining each of the twelve standard deviations one degree of freedom was used, so that the average was determined on the basis of $n-12$. The standard deviation thus determined measures the variability of the herd due to factors other than age. The variability due to age is then given by the difference between the total variance for the group and the variance in which age effects are eliminated.

Using the measure of age variation for the herd as an estimate of the age variation for a particular cow, the variance in the lactations of individual cows due to other factors than age will be given as before, by the difference between the total variance between lactations, given in the previous section, and the variance due to age.

This makes possible, then, the determination of variability with age eliminated, for the herd and for the individual cows. The variance between cows of constant age will, therefore, be given by the difference between the variance of the herd and the variance of the lactations of the individual cow when the variation due to age is eliminated in both cases.

Comparison will show that the standard deviation between cows is the same before and after the variability due to age has been eliminated. This is because the age distribution of the lactations is so similar that when we get an average e^b for the cow, this figure pertains to cows of approximately the same age and therefore has the effect of holding age constant.

Study of the results in Table XI also shows that the effect of age on the lactations of an individual cow is slight for the e^b constant, but is proportionally greater for the a constant. Since there is a well-established effect of age on total yield, this latter fact might be expected. The variability in e^b of cows of the same age is 0·010, while the variation due to factors other than age for a cow as a unit is 0·036 when she had six lactations. For seven lactations, these same figures are 0·017 and 0·035 and for eight lactations they are 0·012 and 0·033. The variability of the initial yield for the same groups is 143 and 193; 167 and 191; 198 and 228. This means that a cow will vary more from lactation to lactation even when age is eliminated as a source of variation than will a group of cows of the same age.

Table XI. *Variability of curve constants due to age.*

e^b for cows having six lactations		a for cows having six lactations	
Average σ for age classes	=0·038	Average σ for age classes	=240·15
$\sigma_{\text{herd 6}}$	=0·039	$\sigma_{\text{herd 6}}$	=260·53
$\sigma_{\text{lactations}}$	=0·038	$\sigma_{\text{lactations}}$	=217·73
$\sigma_{\text{due to age}}$	=0·010	$\sigma_{\text{due to age}}$	=101·00
$\sigma_{\text{lactation-age}}$	=0·036	$\sigma_{\text{lactation-age}}$	=192·88
$\sigma_{\text{b.c.-age}}$	=0·010	$\sigma_{\text{b.c.-age}}$	=143·06
e^b for cows having seven lactations		**a for cows having seven lactations**	
Average σ for age classes	=0·039	Average σ for age classes	=253·98
$\sigma_{\text{herd 7}}$	=0·039	$\sigma_{\text{herd 7}}$	=264·62
$\sigma_{\text{lactations}}$	=0·035	$\sigma_{\text{lactations}}$	=204·91
$\sigma_{\text{due to age}}$	=0·003	$\sigma_{\text{due to age}}$	= 74·24
$\sigma_{\text{lactation-age}}$	=0·035	$\sigma_{\text{lactation-age}}$	=190·98
$\sigma_{\text{b.c-age}}$	=0·017	$\sigma_{\text{b.c.-age}}$	=167·42
e^b for cows having eight lactations		**a for cows having eight lactations**	
Average σ for age classes	=0·035	Average σ for age classes	=302·11
$\sigma_{\text{herd 8}}$	=0·041	$\sigma_{\text{herd 8}}$	=320·72
$\sigma_{\text{lactations}}$	=0·040	$\sigma_{\text{lactations}}$	=252·42
$\sigma_{\text{due to age}}$	=0·023	$\sigma_{\text{due to age}}$	=107·66
$\sigma_{\text{lactation-age}}$	=0·033	$\sigma_{\text{lactation-age}}$	=228·30
$\sigma_{\text{b.c.-age}}$	=0·012	$\sigma_{\text{b.c.-age}}$	=197·85

One might suppose that a cow that had had eight lactations would be more consistent in its lactation curve than a cow with six lactations. Such effect does not seem to be pronounced here, however. The standard deviations for the e^b constant do decrease very slightly, 0·036, 0·035, 0·033, while the standard deviations for the a constant vary 193, 191, 228, for six, seven, and eight lactations, respectively.

In this connection it may be said that there is no evidence of decreased variability in 8-months' total yield for the groups of cows having eight lactations, the figures being, respectively 1250·2, 1178·0, and 1263·7 for six, seven, and eight lactations. Whatever selection was taking place in this herd for the time these records cover, does not seem to be based on the continued breeding of fixed individuals.

Thus the variability of the individual milk yield curves is seen to be due to many factors of which age is by no means the principal one.

SUMMARY.

This study treats of the progress in the milk production of a cow during a single lactation, and the relation of this trend to various factors associated with the particular lactation. The analysis is based on the monthly milk production records of all cows having six or more lactations, in a single large herd of pure-bred Jerseys. The herd provided records on ninety-nine such cows having a total of 679 lactations. Since the trend in the amount of milk produced in a lactation may be described by a simple exponential curve, this equation was fitted to the first 8-months' production records for each of the 679 lactations. From these curves, constants were obtained which measure for each lactation: the theoretical initial yield; the decline of milk yield with time, or in other words the persistency rate; and the scatter of the observed monthly yields about the smooth curve. It is these constants which were studied relative to certain general and biological factors.

Distributions of these three constants are not far from normal and their association is studied by means of the correlation coefficient. There is a fair-sized negative correlation between the persistency factor and initial yield, that is, more persistent milkers tend to start their lactations relatively low. There is no correlation between scatter of the observed monthly yields about the fitted line and either persistency or initial yield.

The trend of the 8-months' total yield, during the years covered by this study, indicates that there has been some selection of cows for a high yield. This may have been brought about by selecting cows with comparatively high initial yield, since such cows have a high 8-months' total. There has been no apparent selection for persistency, and the variability of the observed yields about the smooth curve increases during the time period studied. Total yield shows a slight negative correlation with both persistency and variability of the monthly yields.

The theoretical initial yield is quite closely correlated with the production during the early part of the lactation, so that it seems fair to say that the amount of milk a cow gives soon after calving will be a fair index of her potential worth.

The trend of milk production during a lactation is related to the season in which the lactation starts. The more persistent milkers begin in the winter months, while the cows with higher than average initial yield start their lactation in the early spring. The deviations of monthly yield from the smooth curve show no seasonal variation.

The age of the cow is associated with the trend of the milk production, in that the younger cows are more persistent and the older cows start their lactations at a higher level and deviate somewhat more widely from the fitted curve.

In this sample with records that cannot get closer than one month to parturition, the length of gestation shows no effect on the trend. The length of the resting period and length of time milked show a relation to all three constants describing the trend of milk production. A resting period before a given lactation of more than eight weeks is associated with an increased initial yield, a decreased persistency, apparently due to the selection for early breeding of persistent cows, and somewhat larger deviations of the monthly yields from the smooth trend. The cows which were milked for a longer time than the average are more persistent, start their production at a relatively low level, and tend to be less variable.

The analysis of variation in trend of milk production from one lactation to another shows that there is a greater variation between lactations of the same cow than between cows, in both initial yield and persistency. This result holds not only for total variation but for the variation remaining after variability due to age has been eliminated. Age differences increase the variation in persistency very little but have more effect on variation in initial yield.

From this study two main facts stand out: first, that the amount of milk that a cow gives at the beginning of her lactation is an important figure diagnostic of her ultimate worth as measured by total yield; and second, that breeding for high-producing cows will not reduce the variability within a herd to a low level unless the individual cows have a greater uniformity than they show in this herd, with regard to the trend of their individual lactations.

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(*Received October 5th*, 1934.)

STUDIES ON THE RELATIONSHIP BETWEEN NUTRITION AND WOOL PRODUCTION OF MERINO SHEEP.

I. THE TECHNIQUE EMPLOYED FOR DETERMINING THE UTILISATION OF FOODSTUFFS AND FOR ESTIMATING THE WOOL PRODUCED OVER SHORT PERIODS BY MERINO SHEEP.

By HEDLEY R. MARSTON.

(From the Laboratory of the Division of Animal Nutrition of the Commonwealth Council for Scientific and Industrial Research at the University of Adelaide, South Australia.)

(With Three Text-figures.)

FEEDING stalls of the Lehmann type, which are generally employed when sheep are used for digestibility trials, are useful if wethers act as experimental animals. The wether, however, is not an ideal subject for investigations in which it is desired to estimate the utilisation of individual nutriments, for the portion of the ration which is retained usually constitutes but a small part of that ingested and, in consequence, when attempts are made to draw up a true balance considerable errors are often encountered through the difficulty of ascertaining the total volume of urine excreted during the feeding period. This uncertainty may be overcome, for if the ewe is used a catheter may be passed into the bladder and the urine completely drained at the will of the experimenter.

The type of metabolism crate and experimental procedure which is described here is the result of the experience of some years with Merino ewes as experimental animals. The cage consists essentially of an iron stall of adjustable length (Figs. 1 and 2) that allows the animal complete freedom to stand or to recline in its normal resting position, while always confining it over the collection grids which completely separate the urine and faeces. The faeces pass through a one-inch mesh wire screen and fall on to a perforated zinc slide inclined at 45°, which is a greater slope than the angle of repose of normal wet sheep faeces. The pellets as they are voided rebound off the slide into a container, while the urine trickles through the perforations and runs down an inclined chute into the collecting vessel. Loss of either urine or faeces is prevented by an enamelled iron back-plate. The feed box is fixed to the adjustable front of the cage which is arranged so that the length of the stall may be varied to accommodate animals of different sizes. The water trough is attached to the side and is likewise adjustable. The grids, screens, chute, etc., are constructed so as to be easily removable for cleaning, and during experimental periods these are washed down each day with distilled water, the washings being collected with the urine, and subsequently all parts of the collection apparatus are sprayed, by means of a "Duco gun" with a 10 per cent. solution of thymol in toluol. This prevents any bacterial decomposition of the urine.

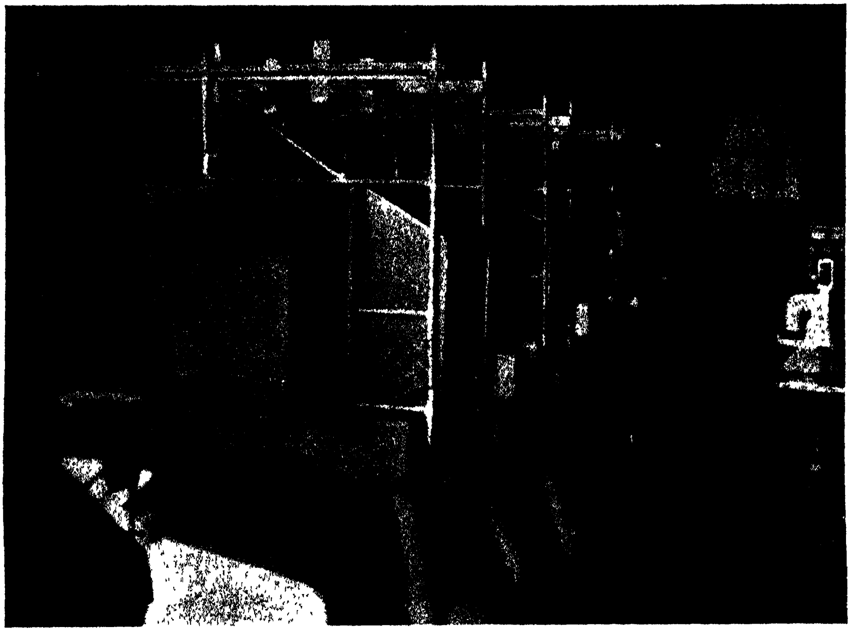

Fig. 1. Side and front elevation of metabolism crate for ewes, showing details of apparatus for separating and collecting the excreta.

The faeces are collected each day and dried completely in a continual draught of air at 90° C. in a highly efficient oven designed for the purpose, from which they are transferred to closed galvanised iron bins and stored until sampled at the end of the period.

During experiments one more cage than the number of animals is employed so as to facilitate the process of cleaning.

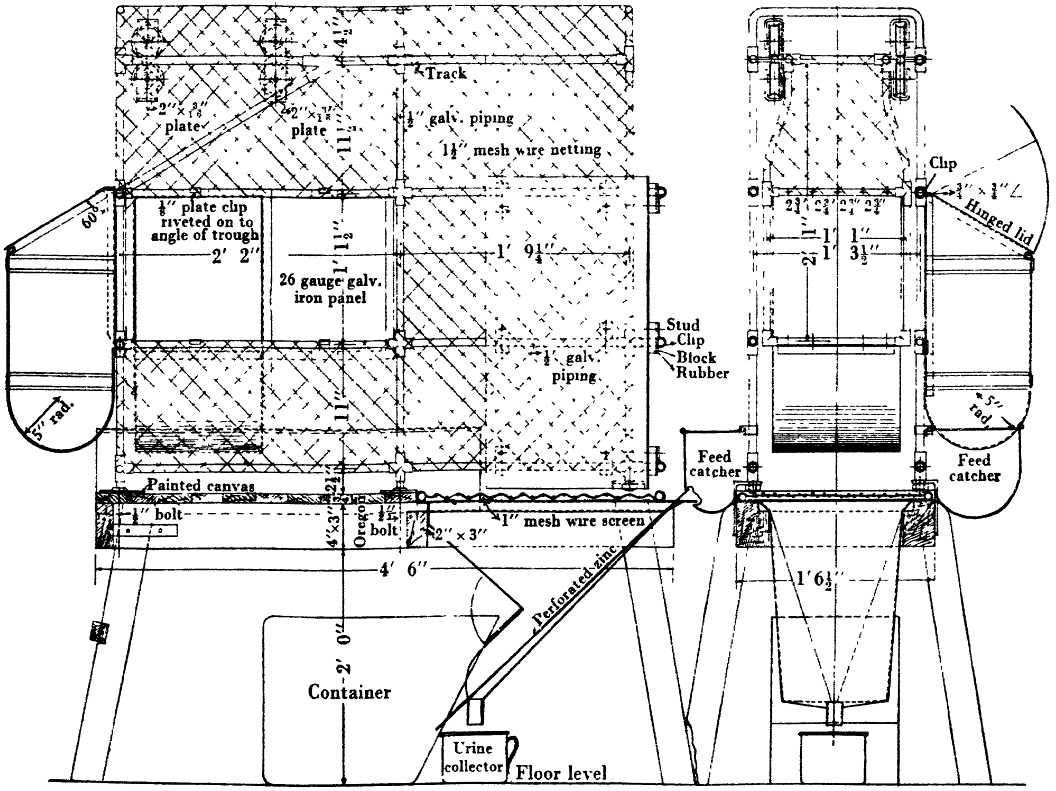

Fig. 2. A section of the cage equipment in the Metabolism Annex of the Nutrition Laboratory, Adelaide.

SAMPLING OF FODDER AND URINE.

The dry, chaffed fodder that is to serve as the basal diet is first mixed as completely as possible in a saucer-like depression of 10 ft. diameter in the cement floor of the sampling room, and this preparation is transferred to a Maclennan mixer from which individual feeds are drawn. The method employed is best explained by describing the actual procedure in an experiment where it was decided to study the influence that an added nutriment had on the nitrogen and sulphur balance and wool growth of a Merino ewe over five periods each of fourteen days. For this about 90 kg. of mixed basal ration was employed. Six kg. lots were transferred to the mixer from which five samples of 1 kg., to be used for the first ration of each period, were drawn into airtight tins. The remaining kg. was collected in a bin. This process was repeated to prepare the series of five matched feeds for each of the fourteen days of the periods. The composite sample in the bin was ground to 50 mesh in a Wiley mill and transferred again into the mixer from which a 2 kg. sample was run into a smaller mixer and from this into a sealed container. The samples, usually 2 gm., for chemical analysis were drawn from this material by quartering. The analyses of individual samples rarely varied more than the limits of chemical methods of examination.

The dried faeces were sampled, ground and treated in the same manner as the fodder. The urine was measured and mixed in a 20-litre bottle from which samples for analysis were drawn direct. It was preserved with toluene and thymol. The amount of faeces passed each day after equilibrium is established is nearly constant, and the experimental error introduced from variation in this path of excretion is of the order of ± 0·4 per cent. for periods of ten days and rather less than half this for periods of twenty days.

By carefully adhering to the procedure outlined above we were able to gauge the intake and excretion of nitrogen and sulphur over periods from ten to twenty days with an accuracy of about ± 1 per cent., and so when the intake of these nutriments was kept low we were able to estimate the retention with considerable precision.

THE CHOICE OF ANIMALS FOR EXPERIMENT.

Mature ewes soon become accustomed to the novel environment of the cage, in which individuals may be confined for many months without any untoward effect.

Although the flock at our disposal is composed of high-producing stud Merinos which have been closely bred and heavily culled for many generations, individual ewes vary in their capacity to convert the nutriments available in the standard diet to wool fleece. The experimental animals were chosen primarily from ewes about three years old, which were proven high producers. These were brought in from pasture and, after a brief preliminary term in pens, confined in the metabolism cages for a probationary period of about six months, during which time they were offered, at noon each day, 1 kg. of basal diet composed of oaten straw chaff two parts, and chaffed lucerne hay one part.

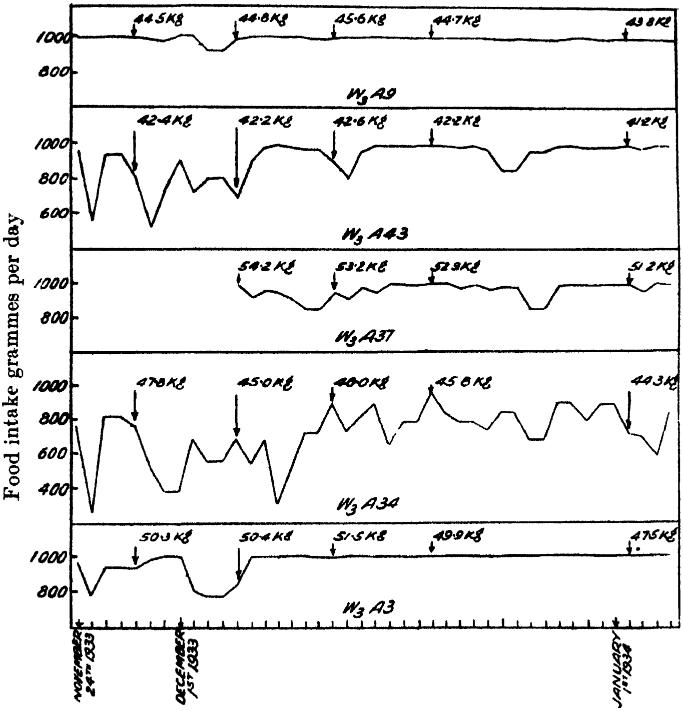

Fig. 3. The daily consumption of basal ration of five Merino ewes undergoing training for metabolism experiments is plotted. The body weight of the individuals is indicated at various points along the time ordinate. Ewe W_3 A 34 was discarded as her capricious intake of food rendered this animal unfit for balance work.

The final choice was made from those animals which maintained steady body weight while completely consuming the ration. The performance of a series of ewes now undergoing training in the Nutrition Annex is indicated in the graph (Fig. 3), which illustrates the effect that individual differences of temperament has on the behaviour of the subjects. W_3A 34, for example, was discarded as her capricious consumption of food rendered this ewe quite unsuitable for further work. The body weight of the heavier animals recedes somewhat under this treatment until complete equilibrium is established, and it is our experience that, once gained, this equilibrium may be maintained for periods up to two years providing the dietary regime is unaltered. Those ewes which become settled in the exacting conditions of the method remain healthy, alert and, at times, even spirited for long periods, and as they are handled every day they become very tame.

THE ESTIMATION OF WOOL GROWTH OVER SHORT PERIODS.

The following technique for estimation of the wool growth over short periods arose from a suggestion of Sir Charles Martin who, during early 1932, pointed out that the investigations into effect of various nutriments on fleece production by the Merino would be facilitated if a direct means of estimating the wool grown during the experimental periods could be devised. He considered that collection of the wool by shaving might be exploited. The method that finally evolved after some twelve months' experimental work is briefly as follows: The wool is directly clipped over an area extending about 25 cm. backwards and 15 cm. downwards from the point of the scapula. Then, having secured the animal in a relaxed position on the operating table, the closely clipped wool is well lathered with good quality shaving soap and completely removed with a half-hollow ground razor which has been carefully honed and stropped. Setting of the razor and the process of shaving must be learned by experience, the criterion of excellence of the operation being judged by the appearance of the skin which should be absolutely free from wool and devoid of any sign of irritation at the completion of shaving. A pink blush is the usual result of careless setting or of uncertain manipulation of the razor. The area from which the wool is to be subsequently collected is then determined from the anatomical points and defined by tattooing. This is best accomplished with the help of a pattern of flexible, transparent celluloid through which slots, sufficiently wide to allow free excursion of the needle, are scribed to form a rectangle 15 × 10 cm. This pattern held lightly but securely on the relaxed, newly shaved skin of the sheep acts as a guide for the tattooing machine which, after adjustment of the needle so as to ensure that the carbon ink will be carried down through the cutis vera, is used to outline the area. Fractious animals should be quietened at this stage by light ether anaesthesia or by a subcutaneous dose of 0·02 gm. per kg. of amytal. Anaesthetics of any sort are to be avoided during the period of experimentation, as these invariably cause some degree of anorexia. The prepared area is thoroughly washed, then dried and protected with saddles made of oblong flaps cut from scoured, full-woolled sheep skins and attached to the experimental animal so that the wool surface fits firmly over the newly shaved skin. This last procedure is employed as a precaution against thermal changes which might presumably influence wool growth through altering the degree of openness of the capillary beds of the exposed surface. Actually, the temperature variations which occurred in the well-insulated annex which housed the animals during the experiments had little, if any, effect on the growth of wool on the exposed areas, even if the protecting saddles were not used.

At the completion of the experimental period the area is washed and the wool removed by shaving up to the edges of the tattooed patch. With care this may be carried out with considerable accuracy. The patch itself is then thoroughly washed with soap and distilled water and clean towels and clipped around so as to prevent contamination of the wool as it is removed. It is again lathered and the experimental crop completely collected, the shavings being washed from the razor into a beaker by means of a jet of distilled water.

When the operation is completed the wool is freed from the superfatted soaps by extracting it on a hardened filter paper with several changes of ether after dehydrating it with absolute alcohol, and it is then scoured with many changes of hot distilled water. The bulk of the water is then removed with alcohol and the wool completely transferred into a tared weighing bottle in which it is dried to constant weight *in vacuo* at 100° C. If the procedure is carefully carried out the small amount of epidermis which is inevitably taken at shaving is not sufficient to effect materially the weight of wool collected.

The relationship that such an experimental crop bears to the amount of fleece produced during the short periods was determined for the series of investigations by slaughtering a number of full-woolled sheep which were closely related to those used in the experiments and directly estimating the ratio that the amount of fibre per sq. cm. on the shoulder patches bore to the total clean scoured fleece, the wool being plucked and treated after submitting the measured skins to the fellmongering process described by Marston and Peirce (5). Seven determinations, although these varied somewhat, led us to conclude that the wool produced per unit area on the shoulder patches was close to 1·4 times that grown per unit area over the whole skin surface of the type of Merino ewe used in this series of investigations. Combining this figure with the equation relating to the body weight and surface area of mature ewes of this flock, discussed fully by Lines and Peirce (4),

$$A = 0{\cdot}117\,W^{0{\cdot}59},$$

where A = skin area in sq. metres,

W = fasting weight in kg.,

we may derive the following expression:

$$F = \frac{S}{1{\cdot}4} \times 0{\cdot}117\,W^{0{\cdot}59} \times 10{,}000$$

or

$$F = 835\,.\,W^{0{\cdot}59} \times S,$$

where F = total amount of wool in gm. grown during experimental period,

S = amount of wool in gm. grown per sq. cm. of the shoulder patch during the period,

W = fasting weight of the animal in kg.

The wool production per unit area on either shoulder usually differed somewhat. This was not unexpected, for the distribution of follicles is not absolutely bilateral and small skin folds often traverse one or other of the circumscribed patches.

The mean of the crops collected from the two shoulder patches was used to estimate the total fleece grown, but the effects of the various treatments on wool growth *was gauged directly from the weight of the crops collected off the areas outlined by tattooing*, and so the argument was not complicated by the assumptions and approximations which are, of necessity, involved in computing the total wool produced in the short periods. However, the total wool produced over such periods derived by this method corresponded quite closely to that which was inferred from the positive sulphur balance and usually accounted for about 85–90 per cent. of the sulphur retained by an animal maintaining constant weight over the experimental periods.

The mean diameter of the shaved fibres was determined by the use of the projection microscope described by Barker and Marsh (1), measurements being taken of twenty fibres in each of one hundred different samples drawn from the dried scoured shavings, two thousand fibres in all being measured for each estimation.

Although alterations in wool production resulted from changes in dietary regime, the process of shaving had little or no influence on the amount of wool grown on the patches during the periods after the animal had been accustomed to the treatment. Nor did temperature changes, providing these were not great, have any measurable effect. These observations substantiate the findings of Trotter (7), Bulliard (2) and Strangeways (6), who were individually led to similar conclusions after careful study of the hair growth of man and the guinea-pig. Study of the effect of nutrition on the growth of hair is, however, complicated by the fact that follicles producing medullated fibres evidence periodical phases of inactivity and differ thus from those producing the non-medullated wool fibres in the Merino sheep which seem to function in a continuous, steady manner providing the animal is supplied with an adequate and fixed ration. Medullated kemp fibres, which are found in certain breeds of sheep are, according to Fraser-Roberts (3), an annual production and, like many hairs, are subject to seasonal variation in growth. This type of fibre was absent from the wool samples taken from our experimental animals.

Exigencies of space prevent recording the complete series of protocols of the early experiments, but the constancy of the wool growth may be gauged from reports in other papers of this series and from data arising from early experiences with ewe $W_2$40, which was often used for subsequent work. This animal was a well-bred, high-producing, strong-woolled Merino, three years of age, and weighing 40 kg. at the beginning of the experiments. She had an equitable temperament and settled down into the exacting regime without evidencing any signs of fretting. At 12 noon each day she was offered 1 kg. of a standard diet, of which she consumed all but a few gm. before two hours elapsed. This ewe was subjected to the rigidly controlled routine for thirty months, during the last two years of which her body weight, exclusive of wool, was practically constant. The following typical data were collected after she had been confined to the metabolism cage for five months. During the latter two months of this period she was accustomed to the experience of shaving.

Table I. *The consumption of basal diet and weight of wool collected from circumscribed shoulder areas of ewe* $W_2$40.

Period	Mean food intake per day	Gm. dry fibre from 15 sq. cm. of shoulder patches	
		Right	Left
A. 10 days	997	1·13	1·20
B. 10 days	986	1·17	1·18
C. 10 days	990	1·12	1·15
D. 10 days	995	1·12	1·16

The maximum variation from the mean wool growth on the areas during this period was about 4 per cent. In the earlier experiments rather larger deviations, up to 6 per cent., were encountered, but after the animals settled down and the operator became more experienced the results from shaving were remarkably constant. The wool growth of $W_2$40, on the basal diet, was about 60 per cent. of that produced while grazing on pastures on the foothills near Adelaide and rather less than half of that possible under ideal nutritive conditions.

ACKNOWLEDGMENTS.

My sincere thanks are due to my colleagues in the Nutrition Laboratory for many points in the design of the metabolism cage. To C. Sleigh I owe a debt of gratitude for the care and training of the experimental ewes, and it is a pleasure to record again my thanks to Sir Charles Martin for his inspiring interest and for his helpful suggestions.

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(*Received July 30th*, 1934.)

STUDIES ON THE RELATIONSHIP BETWEEN NUTRITION AND WOOL PRODUCTION OF MERINO SHEEP.

II. THE EFFECT OF THE ADMINISTRATION OF CYSTINE, CYSTEIN, SULPHUR AND OF METHIONINE ON THE GROWTH OF WOOL OF A MERINO EWE ON A PROTEIN-POOR RATION.

By HEDLEY R. MARSTON.

(From the Laboratory of the Division of Animal Nutrition of the Commonwealth Council for Scientific and Industrial Research at the University of Adelaide, South Australia.)

INTRODUCTION.

THE contrast between the 13 per cent. of cystine in wool keratin and the 0·3–4 per cent. found in natural proteins led Marston and Robertson (17) to suppose that when the protein in the pastures was low the amount of available cystine would impose a limit to the production of wool by the sheep grazing thereon, for it was generally supposed that the animal organism is unable to synthesise this amino acid.

This view has been questioned by King (11), Rimington and Bekker (25) and Fraser and Roberts (2) on the ground that the consumption of cystine from some pastures and from certain experimental diets did not appear to be sufficient to furnish that contained in the wool grown. Woodman and Evans (35) and Pollard and Chibnall (24), on the other hand, reckon that an ordinary English pasture would supply an English breed of sheep with the amount of cystine required to produce an average fleece.

Rimington and Bekker (25) suggest that the difference in their computation between the amount of cystine ingested and the output in the form of wool is derived from proteins formed by the bacteria in the rumen. Fraser and Roberts (3) attribute it to synthesis by the animal which they suggest occurs in the wool follicles.

Neither the argument for nor against an adequate supply of cystine from pasture is convincing. Computations of the amount of cystine that a sheep ingests are subject to error from three causes. There is uncertainty both as to the amount the animal eats and what it eats. Sheep being selective feeders, an analysis of the mown pasture may be quite misleading. These two uncertainties can be removed under experimental conditions, but uncertainty as to the amount of cystine in the fodder remains, for the determination of cystine in grass and hay is, as shown by Lugg (12), not possible, as much of it is destroyed during hydrolysis of the proteins in presence of carbohydrates. Further, the efficiency with which the cystine derived from the proteins of the food is utilised to make keratin is quite unknown and will obviously vary with the nutritional state of the animal and its diet.

The nearest approach to a determination of the amount of cystine in grasses is that made recently by Pollard and Chibnall (24). They extracted the proteins prior to hydrolysing them. From the percentage of nitrogen in these proteins, they still contained a considerable quantity of adherent carbonaceous material, and in computing the amount of cystine in them this was allowed for. The cystine found varied with the species of grass from which the proteins were derived from 0·24 per cent. in cocksfoot to 0·93 per cent. in one of the fescues. Pollard and Chibnall point out that these figures are minimal ones, but, if they are approximately correct, it is difficult to see how a Merino could secure sufficient cystine for optimal growth of wool except from rich pastures. Assuming the mean between the above figures as representing the percentage of cystine in the proteins of a pasture, to produce 5 lb. of clean scoured wool containing 10 per cent. of moisture per year would require an average daily intake of about 120 gm. of true protein, even if the whole were assimilated and all the cystine contained in it transformed into wool. Only exceptional paddocks in Australia would furnish this amount.

An experiment carried out by my colleague Peirce (21) on Merino sheep seems to afford evidence that there may be more cystine in the wool grown than can be accounted for in the food.

Peirce's experiment was designed to ascertain whether the administration of elementary sulphur increased the growth of wool and, for this purpose, the animals were put on a diet on which it was anticipated that the production of wool would be well below the maximum. A diet was chosen in which the digestible true protein was, for the most part, casein because of the low percentage of cystine in this protein. The diet consisted of 420 gm. of chaffed wheat straw, 240 gm. of chaffed wheaten hay, 100 gm. of molasses, 50 gm. of casein and 15 gm. of linseed oil per day. The ration contained approximately 62 gm. digestible crude protein, 430 gm. total digestible nutriments, 1500 calories of metabolisable energy, and was about the maintenance requirement for the adult sheep used when living in pens. The experiment lasted 223 days, during which time both the sheep receiving extra sulphur and the others produced, on an average, 1·34 kg. of clean scoured wool, which would be nearly at the rate of 5 lb. for a year. Allowing 18·25 per cent. for moisture, this amounts to 4·87 gm. of keratin containing 0·63 gm. of cystine per day. Allowing that the 50 gm. of casein contained 0·15 gm. of cystine, this leaves 0·48 gm. to be accounted for. The "digestible crude protein" in the straw, hay and molasses was reckoned to be 12 gm. so that, unless the sheep obtained some cystine besides that contained in the ration, the "crude protein" *which appeared to be assimilated* from the roughage and molasses contained at least 4 per cent. of cystine, which is highly improbable.

Of the two means whereby a sheep might secure a further supply of cystine for the production of wool which have been suggested, the surmise of Fraser and Roberts (3) that some of the tissues of the animal may elaborate cystine appears to me, from analogy with observations on the growth of body and hair in other animals, to be unlikely, but the view espoused by Rimington and Bekker (25) to account for an excess of cystine in wool over that in food is at first sight more attractive.

That ruminants derived protein from non-protein nitrogen through the propagation of bacteria in the paunch was first suggested by Zuntz (36) to explain why asparagine had a "protein-sparing" action when given to ruminants, but not when given to dogs. Although the protein-sparing action of amides has been generally accepted, their capacity to sustain growth has been controverted. From an experiment by Voltz (32), however, it would appear that this, too, can happen. Voltz fed lambs 9 months old upon a diet consisting of starch, sugar, Beckmann's extracted straw (containing 3·1 gm. of nitrogen) and 30 gm. of urea. Most animals would not continue to eat enough of the diet, but he induced one of them to consume it for 8 months on end, during which time it gained 40 per cent. in weight. This animal was evidently an exceptional one, for attempts were made by Morgen, Schöler, Windheuser and Ohlmer (19) and by Scheunert, Klein and Steuber (26) to repeat Voltz' experiment, but without success. In both cases the animals lost weight although they might show a positive nitrogen balance. In Scheunert's experiments this could not be attributed to the growth of wool for this was negligible, but was accounted for by the large amount of urea secreted in the sweat.

Scheunert and his colleagues are at a loss to explain the improvement in appetite, increased digestion of cellulose and protein-sparing action which occurred in their experiment, as in those of Voltz, but they conclude from them that the theory that urea may be converted into bacteria in the paunch and thus supply useful protein may be put aside.

However, vast numbers of bacteria and Protozoa are produced in the rumen, and in so far as they are formed from non-protein nitrogen and are subsequently digested they would make a contribution to the animal's supply of protein, but what the magnitude of this may be is at present unknown. Meantime, it is well to remember that it takes 10^{12} bacteria of average size to weigh a gram, when dry. The nutritive value of bacterial proteins extracted from a mixed culture of organisms from the rumen was examined by Müller (20). He was surprised to find they were not poisonous and were utilised by a dog to the same extent as "blood albumen." The experiments of Hansen and Henriques (5) are more pertinent. They fed young rats on a diet in which the bacteria from a culture on an asparagine medium furnished the whole of the nitrogen. The bacteria were merely washed and dried. They compared the growth of the rats to which the dried bacteria were given with that of others on an identical diet, except that the nitrogen was furnished by dried yeast. Their observations indicated that the nutritive value of the dried bacteria was not inferior to that of dried yeast. Although it may be taken as proven that some bacteria may be a serviceable source of protein, the extent to which, under usual conditions of feeding, nitrogen of biological value is furnished to the animal by the propagation of microbes in its rumen is still uncertain. Whilst protein produced in this way may be important to ruminants on a diet containing a minimal quantity of true protein but abundance of amides, it is yet doubtful whether, when proteins are present in ordinary amounts, the new protein formed exceeds that destroyed by the bacteria.

The experiments presently to be described in this paper were not designed to find out whether a sheep were entirely dependent upon the cystine of the proteins in its food or how it might supplement its supply of this amino acid, but to ascertain the response in wool growth following the administration of additional cystine and cystein and other forms of sulphur to an animal maintained upon a diet on which its wool production was well below the maximum of which it was capable. To achieve a minimum growth of wool and at the same time maintain the animal in physiological equilibrium, a diet low in protein was chosen. This choice was made both on general principles and because, according to Kellner (10), experiments have shown that of those rations which do not suffice for the maintenance of the animal, those which contained sufficient protein had the least unfavourable influence upon wool growth.

Recently, Fraser and Roberts (3) and Fraser and Nichols (4) have, from the results of two series of experiments made by them, come to doubt this doctrine and to conclude that the importance hitherto attached to protein for wool growth has been exaggerated. After scrutinising the records of their experiments, I cannot see that they were justified in arriving at this conclusion from them.

The experiment of Fraser and Roberts was undertaken to test the influence of a supplement of protein, that of Fraser and Nichols one of carbohydrate, on the quantity and quality of the wool grown. In the former experiment, lasting 148 days, no significant difference was found, either in increase of body weight or in the quantity or character of the wool, between those animals on the basal diet and those which received an extra ration of 1½ oz. of digestible protein per day. On the other hand, in the experiment of Fraser and Nichols, the lambs to the daily ration of which 200–500 gm. of maize starch was added, showed after 154 days ten times the increase in body weight and there was an estimated average increase of 62 per cent. in the weight of their clean fleeces.

It would not be just to conclude from these experiments—nor do the authors of the papers—that carbohydrates increase the growth of wool while proteins do not. Fraser and Roberts were satisfied that their control group of sheep were receiving "a diet which was definitely protein deficient." As, however, both groups added 54 per cent. to their weight between 9 and 14 months of age and the one group failed to respond to an additional 1½ oz. of digestible good protein, the protein ration of the controls was presumably sufficient. They do not seem to have determined the amount of protein in the constituents of their diets but, by recourse to Henry and Morrison's tables, it would appear that their control group received a liberal supply of metabolisable energy and 0·23 lb. of digestible crude protein per day. This is about the optimum amount for fattening lambs of the weight of their animals which was arrived at by Kellner (8) from German, and by Bull and Emmett (1) from American experience. Moreover, the nitrogen of Fraser and Roberts' basal ration was mostly true protein and derived from six different sources, which should enhance its value.

The absence of any increase in the wool grown may be interpreted in a similar way, for it is an old observation that rich feeding does not cause a greater growth of wool than a sufficient ration (Kellner (10)).

In Fraser and Nichols' experiment the hoggets were two-thirds of the weight of those of Fraser and Roberts and apparently about 8 months old at the beginning of the experiment. The amount of digestible crude protein in the basal diet appears to have been 0·23 lb., which is again the optimal amount according to Bull and Emmett(1) and rather less by Kellner's standards. The starch equivalent of the diet, however, was only 0·65 lb. in the controls and 1·5 lb. in the animals receiving the supplement. The lambs on the basal ration gained little or lost weight, whereas those whose diet was supplemented by 0·85 lb. of starch increased their weight 57 per cent. in 154 days and it was estimated that they produced 62 per cent. more wool.

The lambs on the basal ration were evidently starved for energy, and of the two interpretations put forward by Fraser and Nichols to account for the difference in the amounts of wool, that one which attributes the small growth on the basal ration to the necessity for the animals to use some of the proteins in their food for fuel seems adequate in the meantime.

Fraser and Nichols consider that the results of their observations have an important practical application. They conclude from them that where animals are losing weight from shortage of food, a carbohydrate supplement may be expected to maintain not only "normal body weight" but "normal wool production" as well. This does not seem to me to be the right conclusion, and I suggest that what the experiments show is that when the ration, which was short of metabolisable energy but contained a sufficient amount of protein, was supplemented with starch the animals grew well and produced 60 per cent. more wool. This might, indeed, have been predicted and also that a supplement of carbohydrate would have improved the growth of body and wool even if they had not been getting quite sufficient protein, for a larger proportion of the inadequate supply would have been available for either purpose. I appreciate that much cannot be expected from a small supplement of protein to the diet of an animal starved for energy, and in order to understand the success which attended a small supplement of dried blood in the experiment at "Meteor Downs" (Marston(14)) it is necessary to comprehend the conditions which obtain in Central Queensland during the winter. At this period, the pasture is standing but dried up and new growth is suspended. There is abundant carbohydrate available, but the protein in the herbage is diminished and, what there is, is less easily digested. In consequence of this small supply of protein and the drain upon it to grow wool in the Merino, the sheep lose their appetite, weaken and will venture less far from water. Hence they starve and the wool growth suffers. A little extra good protein, although its energy value may be small, breaks this vicious circle.

EXPERIMENTAL.

The experimental methods were those described by Marston (16) in the previous paper in this *Journal*. The ewe was one from the Division's high-producing, strong-woolled Merino flock. She settled down to life in a metabolism cage without trouble and became very tame, submitting quietly to handling and shaving.

The daily ration consisted of 1 kg. of a mixture of two parts of chaffed oaten straw and one of chaffed lucerne hay. It contained about 65 gm. of crude protein, about 40 gm. of digestible crude protein (reckoned by the conventional method) and a total energy of 3·9 therms, of which, on an average, 1·95 therms were passed in the faeces and 0·09 in the urine. It had been found by previous experiments that Merino ewes of about 40 kg., when kept in pens, maintained their weight on this diet and produced about 60 per cent. of the wool they usually grew on pasture and rather less than 50 per cent. of their full capacity.

The chemical composition of the ration used in each group of experimental periods varied somewhat owing to the fact that different samples of oaten straw and lucerne hay were used to compound the diet. However, the diet was never changed during any one series of collection periods, and when change was made the animal always received the ration at least 14 days before the collection of the excreta was commenced.

The effect following the addition of 1 *gm. per day of laevo-cystine to the basal diet of ewe* $W_2 40$.

It will be seen from Table I that when 1 gm. per day of laevo-cystine was added to the basal ration the nitrogen retention of the ewe was favourably influenced, the change being brought about mainly by the excretion of less nitrogen in the urine. Five-sixths of the sulphur in the cystine given was absorbed. The increased excretion of this element in the urine appeared mainly in the inorganic sulphur fraction, the neutral sulphur remaining extraordinarily constant over both periods. The faeces passed during the period when cystine was fed contained a little more nitrogen and sulphur.

The wool growth was increased from 2·22 gm., grown over the 20-day basal period on the circumscribed area of 150 sq. cm. on the right shoulder, to 2·54 gm. after the addition of the amino acid to the ration. The mean fibre diameter, however, was not significantly changed, being $23{\cdot}7 \pm 0{\cdot}081\,\mu$ for the basal period and $23{\cdot}9 \pm 0{\cdot}072\,\mu$ for the period during which cystine was fed. The sulphur retention was apparently increased to a greater extent than would be inferred from the observed change in wool growth.

Table I. *The effect on nitrogen and sulphur balance and the wool growth following the addition of* 1 *gm. of cystine per day to the basal ration of ewe* $W_2$40.

	Daily intake from basal ration				
Total (gm.)	Therms	Inorg. S (gm.)	Org. S (gm.)	Total S (gm.)	N (gm.)
1000	3·90	0·37	1·01	1·38	9·86

		Mean daily excretion					
Period	Duration (days)	N in urine (gm.)	N in faeces (gm.)	Total N (gm.)	S in urine (gm.)	S in faeces (gm.)	Total S (gm.)
A. Basal	20	4·55	4·19	*8·74*	0·298	0·814	*1·12*
B. Basal + 1 gm. cystine per day = 0·26 gm. S	20	3·93	4·52	*8·45*	0·402	0·865	*1·27*

	Daily balance and wool growth						
Period	Ingested N (gm.)	Excreted N (gm.)	Balance N (gm.)	Ingested S (gm.)	Excreted S (gm.)	Balance S (gm.)	Comparative wool growth
A	9·86	8·74	+*1·12*	1·38	1·12	+*0·26*	100
B	9·97	8·45	+*1·52*	1·64	1·27	+*0·37*	114

The influence of adding 1 gm. of cystine to the daily diet on the amount of wool grown was less than expected from experience of the effect of increasing the protein in the ration. The small response might be attributable either to destruction of the free cystine by bacteria or because some other constituent necessary for the making of wool was lacking or, as Fraser and Roberts (3) have suggested, be due to depression of the activity of the wool follicles if an animal is kept on a low plane of nutrition. To test these possibilities, an experiment was made in which the amino acid was introduced subcutaneously, thus, avoiding destruction by bacteria in the rumen or gut. Cystein was used instead of cystine as it is soluble in neutral solutions.

The effect following the subcutaneous injection of 1 *gm. of cystein per day into ewe* $W_2$40 *on basal diet.*

1·3 gm. of pure laevo-cystein hydrochloride was dissolved in 5 c.c. of boiled distilled water, the reaction adjusted to *p*H 7·4 with 10 per cent. sodium carbonate, and the volume made up to 10 c.c. This was injected at once into the subcutaneous tissues about the "watershed" of the circulation of the abdomen, the site being previously shaved and sterilised.

Table II. *The influence on nitrogen and sulphur balance and the wool growth which supervened on injection of cystein in ewe* $W_2$40.

	Daily intake from basal ration for all periods				
Total (gm.)	Therms	Inorg. S (gm.)	Org. S (gm.)	Total S (gm.)	N (gm.)
1000	3·90	0·60	1·16	1·82	10·75

		Mean daily excretion					
Period	Duration (days)	N in urine (gm.)	N in faeces (gm.)	Total N (gm.)	S in urine (gm.)	S in faeces (gm.)	Total S (gm.)
A. Basal	10	4·69	4·85	*9·54*	0·450	1·090	*1·54*
B. Basal + 1 gm. cystein injected	10	3·98	4·80	*8·78*	0·462	1·105	*1·56*
C. First post-cystein basal	10	4·18	4·80	*8·98*	0·455	1·140	*1·59*
D. Second post-cystein basal	10	4·30	4·75	*9·05*	0·452	1·100	*1·55*
E. Third post-cystein basal	10	4·52	4·83	*9·35*	0·449	1·105	*1·55*

	Daily balance and wool growth						
Period	Ingested N (gm.)	Excreted N (gm.)	Balance N* (gm.)	Ingested S (gm.)	Excreted S (gm.)	Balance S* (gm.)	Comparative wool growth
A	10·75	9·54	+*1·21*	1·82	1·54	+*0·28*	100
B	10·75	8·78	+*1·97*	1·82	1·56	+*0·26*	134
C	10·75	8·98	+*1·77*	1·82	1·59	+*0·23*	130
D	10·75	9·05	+*1·70*	1·82	1·55	+*0·27*	118
E	10·75	9·35	+*1·40*	1·82	1·55	+*0·27*	107

* Balance of nitrogen and sulphur subsequent to first basal period (A) are exclusive of the 1·1 gm. nitrogen and 2·6 gm. sulphur of the cystein injected in period B.

The nitrogen balance, after the injection of the cystein, was materially benefited (Table II) and the wool production increased about 34 per cent. above that grown on the immediately preceding basal period. Study of the nitrogen and sulphur balance and wool growth over the 30 days subsequent to the completion of the period when the cystein was injected showed that the effect did not cease abruptly on discontinuing the injections, but both nitrogen balance and wool growth were benefited for a further period.

Calculation of the recovery of the sulphur injected as cystein (Table III) indicated that 1·7 gm. of the 2·6 gm. of extra sulphur, introduced into the animal by this means during 10 days, appeared in the increased growth of wool. During the injection period and subsequent 30 days (Table II) approximately 0·9 gm. of extra sulphur appeared in the excreta. The whole of the cystein sulphur was thus accounted for. These calculations are subject to the additive analytical errors of several determinations, but it is apparent that the larger proportion of the injected cystein was utilised for wool growth.

The continued response after the injections had ceased is not unlikely, due to the rapid conversion of the soluble cystein to the relatively insoluble cystine by the mild oxidative environment of the subcutaneous tissues. Cystine may thus have become deposited around the injection site and slowly absorbed from there during the post-injection periods.

A steady leakage of the amino acid into the circulation would constitute ideal conditions for its utilisation, for if the absorption were slow

Table III. *The recovery of injected cystein in the wool of ewe* $W_2$40.

Period	Computed wool keratin per day (gm.)	S in fleece (gm. per day)	S in fleece above basal for period (gm.)
A. Basal	5·8	0·205	—
B. Basal + cystein	7·7	0·270	0·65
C. First post-cystein basal	7·5	0·260	0·55
D. Second post-cystein basal	6·8	0·240	0·35
E. Third post-cystein basal	6·2	0·220	0·15
Total sulphur in wool above basal			1·7
Sulphur injected as cystein			2·6
Percentage recovery of cystein in wool			66

Table IV. *Distribution of sulphur in urine of ewe* $W_2$40.

Period	Duration	Total S (gm.)	Total sulphate S (gm.)	Inorganic S (gm.)	Conjugated S (gm.)	Neutral S (gm.)
A. Basal	10 days mean	0·450	0·390	0·017	0·373	0·060
B. Basal + 1 gm. cystein per day	10 days mean	0·462	0·386	0·020	0·366	0·076
C. First post-cystein basal	1st day	0·470	0·400	0·024	0·376	0·070
	2nd day	0·460	0·396	0·023	0·373	0·083
	3rd day	0·465	0·390	0·023	0·367	0·075
	4th day	0·450	0·384	0·024	0·360	0·066
	5th day	0·440	0·360	0·020	0·340	0·080
	Mean 6th–10th day	0·443	0·360	0·019	0·341	0·083
	Mean for C period	*0·455*	*0·374*	*0·022*	*0·360*	*0·076*
D. Second post-cystein basal	10 days mean	0·452	0·388	0·021	0·367	0·064
E. Third post-cystein basal	10 days mean	0·446	0·380	0·019	0·365	0·066

it is not unlikely that the follicles could utilise the cystine rapidly enough to prevent its accumulation in the blood and so avoid its destruction by oxidative deaminisation. The alternative possibility that the cystine was transported and stored elsewhere is more unlikely, although the balance data in the previous experiment suggested that some measure of storage resulted when cystine was fed. The intermediate behaviour of the injected cystein is being further investigated.

Table V. *Influence of cystein injections on the distribution of fibre diameter of wool grown on circumscribed area on left shoulder of ewe* $W_2 40$.

Diameter of fibre	Number of fibres				
μ	Period A	Period B	Period C	Period D	Period E
16	152	133	112	126	166
18	274	165	168	238	249
20	283	278	290	240	252
22	325	349	360	287	289
24	361	400	389	448	391
26	196	220	224	190	189
28	153	121	154	140	134
30	102	138	135	131	152
32	63	62	68	65	58
34	35	59	52	60	44
36	75	77	74	87	84
Totals	2019	2002	2026	2012	2008
Mean diameter	*23·38 μ*	*23·99 μ*	*24·00 μ*	*23·99 μ*	*23·56 μ*
P.E.	±0·0742	±0·0742	±0·0722	±0·0782	±0·0775
Coefficient of variation %	21·2	20·4	20·0	21·8	21·9
Weight of wool mg./sq. cm./day	*0·76*	*1·02*	*1·00*	*0·89*	*0·81*
Comparative wool growth	100	134	130	118	107
Comparative length of fibre	100	127	126	110	97

The increase in the weight of wool grown on the circumscribed shoulder area subsequent to the injections of cystein was accompanied by a significant increase in the fibre diameter, but this increase was not sufficient to account for the extra weight which may only be explained by an increase in length growth of the individual fibres. This was great enough to be apparent on observing the patch prior to collection of the wool. The data relating to the wool measurements are set out in Table V.

The effect of adding elementary sulphur to the basal diet.

In this experiment, 1 gm. of finely divided elementary sulphur was added to the daily basal ration. No attempt was made to draw up a final sulphur balance for any sulphur rejected as dust at the bottom of the food bin would render this unreliable.

Five periods, each of 10 days, were studied, sulphur being added to the ration during the third period. From Table VI it will be seen that no significant increase in wool production or any increase in nitrogen retention occurred, although about 40 per cent. of the extra sulphur fed appeared in the sulphate fraction of the urine, a large proportion of it being paired as ethereal sulphate. About 15 days after the ingestion of the sulphur had ceased the ewe appeared unwell and refused to eat the whole of the ration which she had steadily and totally consumed for over 10 months. She soon recovered, but the nitrogen balance for this period was considerably altered and both nitrogen retention and wool growth were decreased. It is doubtful, however, whether the ingestion of sulphur had anything at all to do with the temporary indisposition of the ewe. The mean fibre diameter of the wool grown over these 50 days was not significantly changed.

Table VI. *The influence of adding* 1 *gm. of elementary sulphur to the basal ration of ewe* $W_2$40.

Total (gm.)	Daily intake from basal ration for all periods Therms	Inorg. S (gm.)	Org. S (gm.)	Total S (gm.)	N (gm.)
1000	3·90	0·50	0·98	1·30	10·80

Period	Duration (days)	Mean daily excretion N in urine (gm.)	N in faeces (gm.)	Total N (gm.)	Balance N (gm.)	S in urine (gm.)	Comparative wool growth
A. Basal	10	4·54	4·84	9·38	*+1·41*	0·390	100
B. Basal	10	4·49	4·90	9·39	*+1·48*	0·385	100
C. Basal + 1 gm. elementary S per day	10	4·55	4·87	9·42	*+1·40*	0·835	98
D. First basal post-sulphur	10	4·59	4·80	9·39	*+1·48*	0·405	96
E.* Second basal post-sulphur	10	4·80	4·90	9·70	*+1·07**	0·396	96

* The animal was obviously sick during the last period and refused on three days to consume the whole of the 1 kg. of basal ration offered.

The effect following the injection of methionine contrasted with that following injection of an equivalent of cystein under identical feeding conditions.

In this experiment the length of the periods was increased to 14 days and the urine collected each day after the first five had elapsed so that the partition of sulphur and nitrogen could be studied. The methionine and cystein were injected at 12 noon on the first 10 days of the respective periods.

The sample of methionine used was isolated from casein by Pirie's first method (23). It was 90 per cent. pure and free from disulphide and sulphhydryl compounds. The quantity of this material, which had the same amount of sulphur as 0·5 gm. of cystein, was dissolved in sterile saline and injected each day as in Exp. 2.

Table VII. *The influence on nitrogen and sulphur balance and the wool growth which supervened on injection of methionine and of cystein in ewe* W$_2$40.

	Daily intake from basal ration for all periods				
Total (gm.)	Therms	Inorg. S (gm.)	Org. S (gm.)	Total S (gm.)	N (gm.)
1000	3·90	0·56	0·95	1·51	10·59

		Mean daily excretion					
Period	Duration (days)	N in urine (gm.)	N in faeces (gm.)	Total N (gm.)	S in urine (gm.)	S in faeces (gm.)	Total S (gm.)
A. Basal	14	4·16	5·15	*9·31*	0·380	0·890	*1·27*
B. Basal + 0·65 gm. methionine per day for first 10 days	14	4·03	5·20	*9·23*	0·445	0·895	*1·34*
C. Basal + 0·5 gm. cystein per day for first 10 days	14	3·50	5·23	*8·73*	0·398	0·890	*1·29*

	Daily balance and wool growth						
Period	Ingested N (gm.)	Excreted N (gm.)	Balance N (gm.)	Ingested S (gm.)	Excreted S (gm.)	Balance S (gm.)	Comparative wool growth
A	10·59	9·31	+*1·28*	1·51	1·27	+*0·24*	100
B	10·63	9·23	+*1·40*	1·60	1·34	+*0·26*	105
C	10·63	8·73	+*1·90*	1·60	1·30	+*0·30*	116

The immediate effect (Table VIII) of the injections suggested that the nitrogen retention was benefited to some extent, but this influence fell away during the latter portion of the period and the final nitrogen balance, although greater than basal, was not as favourably increased as would be inferred from the early observations on the urine. Most of the sulphur injected as methionine appeared in the sulphate fraction of the urine, largely as ethereal sulphates. The neutral sulphur fraction was, however, increased. The wool growth was apparently augmented about 5 per cent., but this is so close to the error of the determination as to render it of doubtful significance.

Table VIII. *Distribution of sulphur in urine of ewe* $W_2$40.

		Daily excretion in urine		
Period	Duration	Nitrogen (gm.)	Total S (gm.)	Sulphate S (gm.)
A. Basal	Mean 14 days	4·16	0·380	0·345
B. Basal + 0·65 gm. methionine per day subcutaneously	Mean first 5 days	3·75	0·458	0·400
	6th day	4·05	0·492	0·414
	7th day	4·20	0·452	0·402
	8th day	4·04	0·475	0·401
	9th day	4·35	0·517	0·417
	10th day	4·03	0·485	0·412
	Mean first 10 days	*4·07*	*0·479*	*0·407*
Methionine injections discontinued	11th day	3·95	0·409	0·345
	12th day	4·02	0·402	0·337
	13th day	4·00	0·389	0·346
	14th day	4·11	0·374	0·339
	Mean for 14 days	*4·05*	*0·445*	*0·381*
C. Basal + 0·5 gm. cystein per day injected subcutaneously on first 10 days	Mean for 14 days	3·50	0·396	0·356

In period C, the injection, under exactly identical conditions, of the same amount of sulphur as cystein was followed by a definite increase in retention of sulphur and nitrogen and the significant increase of 14 per cent. in wool growth (Table VII). The mean fibre diameter of the wool collected from the shoulder patch was unchanged during the period of the experiment, being 23·56 ± 0·080 μ for the basal period, 23·60 ± 0·084 μ for the methionine period, and 23·60 ± 0·083 μ for the cystein period.

DISCUSSION.

It will be seen from the preceding tables that the intake of nitrogen in the daily basal ration varied in different experiments from 9·86 to 10·90 gm. and the total sulphur from 1·30 to 1·82 gm. The variation of the sulphur could be largely accounted for by the difference in the inorganic sulphate fraction. The wool production on the circumscribed shoulder area of the experimental ewe during different periods on the basal ration varied between 0·76 and 0·80 mg. per sq. cm. per day, but no direct correlation could be established between the total sulphur intake and the wool growth. The intake of organic sulphur was approximately four times that bound in the wool fleece. The utilisation of the crude protein of the diet would appear to be greater than that derived from the difference between the nitrogen ingested and that in the faeces. The metabolic end-products secreted into the gut may be expected, if the method of assessing them advocated by Pfeiffer (22) is adopted, to account for 1·6 gm. of nitrogen, or if that recommended by Morgen, Berger and Westhauser (18), who subtract all that nitrogen of the faeces which is brought into solution by tryptic as well as peptic digestion from the total nitrogen of the faeces before reckoning the coefficient of digestibility, to account for 3·4 gm. The same applies to the utilisation of sulphur, a large amount of the organic sulphur in faeces having come from the bile and the chondroitin sulphuric acid in the mucus. Practically all of the sulphur and over 85 per cent. of the nitrogen could be rendered soluble by treating the finely divided chaffed lucerne hay and oaten straw with enzyme preparations, isolated from pancreas and intestinal mucosa by adsorption on kaolin and on Tonerd Cγ of Willstätter and Krause, so that it is likely that the digestion of the crude protein was nearer 80 per cent. than the 60 per cent. arrived at by the conventional method.

The basal ration, with less than 11 gm. of nitrogen, contained sufficient protein to maintain physiological equilibrium of the animal and furnish a growth of wool of about 60 per cent. the amount it would do on pasture. By the means described in the previous paper on experimental methods (Marston (16)), it is computed that the ewe was able, in some way, to secure 0·75 gm. of cystine daily for wool production. The crude protein in the ration was 66 gm. and, assuming 50 gm. to be digested, this must have contained at least 1·5 per cent. cystine if all of this amino acid required for making wool was preformed in the food. Whether the proteins in the lucerne hay and oaten straw contain so much must remain doubtful until satisfactory methods for isolating them and determining cystine in them are discovered.

Whether the ewe on the basal ration was deriving cystine from some other source than that in the food or not, the response to injections of cystein shows that she was not supplementing the supply sufficiently for optimal growth of wool, and that even with 11 gm. of nitrogen in her diet she could produce 30 per cent. more wool, and at this rate would produce an annual fleece containing over 7 lb. of clean scoured wool.

The experiment in which sulphur was added to the ration was undertaken in view of the observations of Steyn (28, 29) at Onderstepoort. Steyn concluded that sheep, to which he was administering sulphur, produced more wool and that the increase seemed to be correlated with the dose given. The experiments were not designed to test the effect of sulphur on the growth of wool. They were, admittedly, in some respects, unsuitable, and the author does not claim that they are conclusive. His groups contained a heterogeneous lot of sheep and the measurement of their variance by the application of Fisher's "*Z*" test to his figures gives a value for *Z* of 0·592 for his first year's observations and 0·467 for his second. For the lowest level of significance—a probability of 0·05—a value for *Z* exceeding 0·613 is required. Peirce (21) repeated Steyn's observations in Adelaide with a more homogeneous group of sheep. The addition of 2 gm. of sulphur daily in the form of brimstone and treacle to the diet of hay, straw, molasses and casein did not increase the wool production compared with controls. Seddon and Chamberlain (27) also failed to observe any effect from administering sulphur to sheep grazing on natural pastures in New South Wales. In the experiment, the results of which are recorded in Table VI, the addition of 1 gm. of elementary sulphur per day to the basal diet, the wool-producing capacity of which was proven to be favourably increased by the addition of cystine, resulted in neither increase in nitrogen retention nor augmented wool growth of the experimental ewe, although bacterial activity in the rumen and lower intestinal levels was sufficient to convert at least 40 per cent. of it into a form which was assimilated by the animal. This was, for the most part, excreted in the sulphate fraction of the urine. As the intake of sulphur in this experiment was approximately doubled without beneficial effect, it is unlikely that the flora of the rumen converted any of it to cystine, or to any other assimilable organic sulphur compound which, under the conditions of the experiment, could save cystine in metabolism.

The effect of injecting methionine on wool growth was studied because, from observations of Jackson and Block (6) and Weichselbaum, Weichselbaum and Stewart (33), it might be inferred that cystine and methionine are interconvertible in metabolism. They found that the growth of rats upon the Sherman-Merrill diet was increased on the addition of methionine to their ration. Arrest of growth on the Sherman-Merrill diet had hitherto been supposed to be entirely due to lack of cystine.

The absence of striking similarity in structural make-up of methionine and cystine renders this unlikely, and, as Jackson and Block (6) point out, the observations can be interpreted by supposing that whilst each of these bodies is indispensable for growth, there is at least one metabolic function which can be cared for by either or by some common metabolite.

The similarity in the changes in sulphur metabolism of dogs when either cystine or methionine is administered with brombenzene, which has been observed by White and Lewis (34), can also be explained if cystine and methionine have some common product of intermediary metabolism which is essential for normal function, and that when excess of methionine is supplied to the diet the cystine present is thereby spared. It is conceivable that cystine was rendered available by this means for production of phenyl-mercapturic acid when methionine was administered together with the monohalogen derivative of benzene in White and Lewis' experiments with dogs, and du Vigneaud's observations (31) that homo-cystine will, like methionine, increase the growth of rats on "cystine-deficient" diets, suggest that this sulphhydryl compound is an intermediate step in the metabolism of methionine.

The investigations of du Vigneaud (30) render it doubtful that methionine can give rise to cystine in the animal organism. In his experiments a nearly cystine-free diet was employed that contained 14 per cent. of casein hydrolysate from which cystine had been precipitated as the silver salt, the histidine and tryptophane destroyed in the process being replaced. The "B" accessory food factors were added as 100 mg. per day of yeast concentrate which contained the only available cystine in the ration. Rats grew well when offered this diet *ad lib.*, supplemented with 20 mg. of laevo-cystine each day, but they rapidly lost weight if the laevo-cystine were removed or replaced by the dextro enantiomorph. As this diet contains approximately twice the amount of methionine usually present in "cystine-deficient" diets one might reasonably expect that it would support some measure of growth if methionine had the capacity to give rise to cystine in the organism.

Injection of methionine into the sheep did not increase the wool growth or sulphur retention to the same extent as an equivalent amount of cystein administered under identical conditions, and the slight increase in wool growth which supervened, if this can be considered significant, might presumably be due to the injected methionine sparing some of the cystine assimilated from the basal ration and thus rendering it available for wool production. The fact that the response of the sheep to injected methionine was small would indicate that the basal diet already contained sufficient of this constituent for the performance of normal metabolic function, and that nearly all of the ingested cystine was being taken for those purposes for which cystine is indispensable.

SUMMARY.

1. The question whether the amount of cystine in the wool grown may exceed that in the diet is discussed.

2. When 1 gm. of laevo-cystine was added to the daily ration of a ewe on a low protein diet, the wool grown upon a circumscribed area was 14 per cent. more than that grown during a similar period immediately preceding. Four-fifths of the sulphur in the added cystine was absorbed, about half of which was excreted in the urine during the period the cystine was being fed.

3. When 1 gm. of laevo-cystein was injected subcutaneously each day for 10 days, a 34 per cent. increase in wool growth occurred. During three succeeding periods, of 10 days each, immediately after the injections had been discontinued, the increases were 30, 18 and 7 per cent. respectively. Of the 2·6 gm. of sulphur in the cystein injected during the 10 days, 1·7 gm. was retained and could be accounted for by the extra amount of wool grown during that and the succeeding periods.

4. When 1 gm. of sulphur was added to the food daily no increase in wool production occurred. Forty per cent. of the extra sulphur in the diet was excreted in the urine of the period during which it was given. There was no increased retention of nitrogen.

5. During the period when methionine was injected a small but doubtfully significant increase in wool growth occurred.

6. The greater weight of wool produced by injections of cystein was due to increase both in length and in the mean diameter of the fibres.

GENERAL CONCLUSIONS.

Whether the sheep is entirely dependent upon the cystine in its food to make wool or not, the administration of cystine to one upon a low protein diet was followed by a material increase in the growth of wool. The increase was trebled when cystein was injected subcutaneously so as to avoid destruction of the free amino-acid by bacteria in the alimentary canal.

Elemental sulphur did not have this effect and the small increase which occurred when methionine was injected was not enough to be significant.

ACKNOWLEDGMENTS.

I am grateful to my colleagues in the Nutrition Laboratory for their co-operation and help. Special thanks are due to Miss F. E. Allen and to Messrs H. Münz, F. C. Farr and C. Sleigh.

We wish also to thank the Directors of the Commonwealth Bank and the members of the Pastoral Research Trust, who have made these experiments possible by grants for equipment.

The work was stimulated by the interest of Sir Charles Martin, and the author gratefully acknowledges his help in the preparation of the manuscript.

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(*Received July 30th*, 1934.)

STUDIES ON CALCIUM CYANAMIDE[1].

V. THE UTILISATION OF CALCIUM CYANAMIDE IN POT CULTURE EXPERIMENTS.

By H. L. RICHARDSON and E. M. CROWTHER.

(*Chemistry Department, Rothamsted Experimental Station, Harpenden, Herts.*)

(With Three Text-figures.)

In the first paper of this series (1) we discussed the chemical aspects of investigations continued for many years in this laboratory on the fertiliser value of "Calcium Cyanamide" (the commercial form of calcium cyanamide, $CaCN_2$). We showed that, contrary to some of the earlier views, the initial stages of its decomposition in the soil, through urea to ammonia, proceed very rapidly and are complete long before the nitrification of the ammonia in ammonium salts added at the same time. The final stage—the production of nitrate—may suffer a delay which is often greater than the time required for the initial decomposition of the cyanamide. This delay was shown by B. K. Mukerji (2) to be due to the toxic action of the cyanamide nitrogen on the nitrifying organisms. The evidence on which these conclusions were based was derived from experiments in flasks and in uncropped pots in the greenhouse, from the early stages of pot experiments with barley, before the plant was large enough to cause disturbance, and, finally, from field experiments.

In the present paper we present the results of further soil analyses conducted throughout the growth of barley and mustard plants, in pot cultures, and endeavour to relate the differences in growth of the crops to the course of the decomposition of the added fertilisers. Pot experiments are particularly suited to such investigations, for they make it possible to compare several soils, under uniform though admittedly artificial conditions, without disturbance from unknown leaching losses. Since, however, the pot culture conditions are so artificial it is in the highest degree unsafe to transfer the results directly to practice.

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[1] Several past and present members of the Rothamsted staff collaborated in the work described in this series of papers and, although it proved impossible to publish the contributions separately, the names of those responsible for the major experiments are given in the text, tables or figures.

Pot experiments provide information which may be used in interpreting the results of field experiments, but actual trials in the field are necessary to decide the practical value of a fertiliser. The results of field experiments on the use of calcium cyanamide as a fertiliser and in weed control, as well as on its effect on germination, are described separately in the *Empire Journal of Experimental Agriculture* (3).

PRELIMINARY EXPERIMENTS IN POTS.

Series of pot cultures comparing ammonium sulphate and calcium cyanamide on barley and mustard were conducted by R. B. Dawson (4) in 1927 and by the late A. J. Walker in 1927. The soils used in these experiments are described on pp. 135–9 together with those for the main experiments. The conditions in these preliminary experiments proved unsuitable for an adequate test, but the results are quoted to illustrate the effects of heavy additions of fertilisers on barley sown too late to allow the nitrogen absorbed to be utilised efficiently. The barley ears formed poorly, the grain/straw ratio was unduly low, and the nitrogen content of the grain was extremely high.

In 1926 it was noted that the plants with calcium cyanamide in the Rothamsted soil tillered more and had more deeply coloured leaves than those with ammonium sulphate. In the Millstone Grit soil at an early stage there were more tillers from calcium cyanamide, but the advantage was soon lost.

The final yields summarised in Table I show a slight advantage of calcium cyanamide over ammonium sulphate in the Rothamsted but not in the Millstone Grit soil. In a mustard experiment with five soils in the autumn of 1926 (Table II) calcium cyanamide gave poorer results than ammonium sulphate in the Millstone Grit soil and in a Fen soil; the two fertilisers were substantially equal in the other three soils. It was noted

Table I. *Pot experiments on barley* (*late sown*) 1926—
R. B. Dawson's data.

Comparison of ammonium sulphate (S) and calcium cyanamide (Cy.), each supplying 0·61 gm. nitrogen per pot of 11·5 kg. soil. Manures added and barley sown April 21; harvested August 23. Soils: Millstone Grit (M.G.) and Rothamsted Hoos Field (R.H.). All data in grams per pot and means of four pots.

Soil ...	Millstone Grit			Rothamsted Hoos		
Fertiliser	No N	S	Cy.	No N	S	Cy.
Dry grain	10·4	17·8	17·3	6·5	16·9	19·3
Dry grain + straw	38·5	58·1	56·3	22·5	52·4	56·2
Nitrogen in grain	0·244	0·477	0·485	0·144	0·325	0·360
Nitrogen in grain + straw	0·379	0·724	0·730	0·221	0·487	0·526

that on the two acid soils (W.S. and M.G.) ammonium sulphate caused more browning and withering of the leaf margins than calcium cyanamide, probably through a slightly greater acidification of the soil.

Table II. *Pot experiments on mustard* 1926—*R. B. Dawson's data.*

Comparisons of ammonium sulphate (S) and calcium cyanamide (Cy.), each supplying 1·22 gm. nitrogen per pot of 23 kg. soil. Manures added and mustard sown September 6; harvested November 25. Soils: Millstone Grit (M.G.), Woburn (W.S.), Leagrave (L.C.), Rothamsted Hoos Field (R.H.) and Fen. All data in grams per pot and means of four pots.

Soil ...	M.G.	W.S.	L.C.	R.H.	Fen
Dry crop					
No N	43·3	34·4	38·7	25·6	38·5
S	49·4	37·0	43·9	39·0	51·0
Cy.	39·9	37·0	46·6	37·8	42·1
Nitrogen in crop					
No N	1·67	1·46	1·13	0·64	1·32
S	2·16	1·83	1·99	1·66	2·02
Cy.	1·97	1·85	1·95	1·58	1·66

Table III. *Pot experiments on barley* (*late sown*) 1927—*the late A. J. Walker's data.*

Comparisons of single and double dressings of ammonium sulphate (S) and calcium cyanamide (Cy.) in pots with 11 kg. soil. Manures added and barley sown March 28–30. In Millstone Grit (M.G.) and Woburn (W.S.) soils, harvested August 13. In Rothamsted Hoos Field (R.H.), Leagrave (L.C.) and Fen soils germination was faulty and barley was resown on April 25 and harvested on September 1. All data in grams per pot and means of four pots.

Soil ...	M.G.	W.S.	L.C. (resown)	R.H. (resown)	Fen (resown)
Dry grain					
No N	12·4	14·6	8·5	5·1	14·0
0·32 N as S	15·2	15·2	12·2	11·2	13·1
0·32 N as Cy.	13·8	17·4	12·0	12·4	13·7
0·64 N as S	16·6	10·9	11·5	9·3	11·3
0·64 N as Cy.	13·1	13·2	10·6	9·9	12·2
Dry grain + straw					
No N	28·2	30·8	20·4	12·7	35·0
0·32 N as S	36·3	36·1	29·9	31·0	34·7
0·32 N as Cy.	35·8	36·7	29·1	31·8	36·5
0·64 N as S	39·2	32·1	29·7	26·6	33·8
0·64 N as Cy.	34·5	34·1	27·3	27·9	34·7
Nitrogen in grain					
No N	0·146	0·188	0·108	0·069	0·206
0·32 N as S	0·307	0·277	0·240	0·192	0·327
0·32 N as Cy.	0·289	0·294	0·246	0·199	0·316
0·64 N as S	0·424	0·302	0·343	0·261	0·366
0·64 N as Cy.	0·330	0·313	0·306	0·369	0·357
Nitrogen in grain + straw					
No N	0·221	0·240	0·148	0·092	0·311
0·32 N as S	0·469	0·391	0·338	0·314	0·503
0·32 N as Cy.	0·476	0·392	0·343	0·376	0·495
0·64 N as S	0·641	0·526	0·512	0·412	0·626
0·64 N as Cy.	0·558	0·498	0·451	0·406	0·608

In a small experiment on mustard on Rothamsted soil, with a very heavy rate of application (1 gm. of nitrogen per pot of 11 kg. soil), R. B. Dawson found that the substitution of half of the nitrogen in calcium cyanamide by dicyanodiamide greatly reduced the yield and halved the nitrogen recovery. Dicyanodiamide equivalent to the added calcium cyanamide or ammonium sulphate reduced the crop to one-sixth and allowed practically no nitrogen recovery in excess of the unmanured pots. The familiar symptoms of dicyanodiamide poisoning—whitening of the leaf margins—were well shown and persisted throughout growth.

The 1927 series (Table III) was set up parallel with the uncropped pots described in the first paper (1) of this series, but, owing to faulty germination, it was necessary to resow three of the soils at a later date. Plant development was poor throughout, and many of the ears failed to emerge before harvest. In four of the five soils the double rate of application of ammonium sulphate gave lower yields than the single rate, and in two of them even lower yields than the unmanured. The double rate of calcium cyanamide gave lower yields than the single rate in all soils, but the differences between calcium cyanamide and ammonium sulphate were small and inconsistent, except in the Millstone Grit soil. The experiment was concerned rather with the harmful effects of excess nitrogen on a late crop (possibly with additional disturbance from dicyanodiamide) than with the normal utilisation of the fertilisers.

POT EXPERIMENTS, 1928–31.

The fact that calcium cyanamide twice gave poor results in the Millstone Grit soil, which was known to nitrify slowly and to exhibit a marked retardation of nitrification with calcium cyanamide, led to its more thorough examination. In 1928, 1929, 1930 and 1931, W. E. Brenchley conducted pot experiments on barley and mustard in the Millstone Grit and other soils, using a uniform technique with early sown barley, and, generally, with much lower rates of application of the nitrogenous fertilisers than in the preliminary experiments. In order to explore a wide range of concentrations of the fertilisers, several rates of application were compared in three of the years.

The soils chosen for this work had widely differing characteristics, as may be seen from their responses to nitrogen and from the mechanical analyses in Table IV. The soil reactions at the end of the experiments have already been given in the first paper of the series ((1), p. 330).

The Fen soil, from Cambridgeshire, was of the *Niederungsmoor* type. It was rich in calcium carbonate as well as in organic matter, and gave so much readily available nitrogen that an extra dressing of nitrogen had little, if any, effect. In consequence, it was not used in the main series.

Table IV. *Mechanical analyses of soils used in pot experiments (on oven-dry basis).*

Soil ...	Fen (1926–7)	M.G.	L.C.	R.B.	W.S.
Coarse sand	19·8	24·4	7·6	7·9	57·8
Fine sand	15·9	32·6	17·3	43·2	24·4
Silt	5·4	16·6	9·4	26·8	5·5
Clay	12·5	18·4	20·8	17·8	6·8
Carbonates	9·1	0·0	33·2	0·1	0·0
Air-dry moisture	20·1	3·2	5·2	3·2	1·6
Loss by solution	3·2	1·2	1·7	0·9	0·7
Difference	+14·0	+3·6	+4·8	+0·1	+3·2
Total	100·0	100·0	100·0	100·0	100·0
Loss on ignition	16·4	6·6	6·9	5·6	3·3

The Millstone Grit soil (M.G.) was an acid loam from the Pennines, near Stalybridge, Cheshire; in its natural state it was too acid for barley. Accordingly, it received additions of calcium carbonate sufficient to raise its pH to about 6·5. The soil had, when limed, considerable reserves of decomposable organic matter which slowly yielded ammonia and nitrate during the growth of the crop; nitrification was begun more slowly than in the other soils. The response to added nitrogen was generally low, and the barley grain produced was rich in nitrogen.

The Leagrave calcareous soil (L.C.), which came from arable land at the foot of the Chiltern chalk escarpment, is to be classified as a *rendzina*. It also proved to be rich in readily available nitrogen, and it nitrified rapidly.

The Rothamsted soil (R.H.) used in 1928 and 1929 was a heavy loam, taken from an area in Hoos Field which had been deliberately impoverished by continued arable cropping without manure. It still contained enough calcium carbonate from applications early last century to be alkaline. It gave low nitrate levels and a marked nitrogen response. The other Rothamsted soil (R.B.) used in 1929 and 1931 came from Broadbalk Field, from a strip adjoining the continuous wheat plots but markedly different from them in composition and behaviour. Until recent years its site was a part of the wide grass headland—the actual "Broad Balk"—which formerly surrounded the wheat field, after the old Hertfordshire custom described by William Cobbett. It was slightly acid and had no reserves of calcium carbonate, but it had considerable reserves of readily available nitrogen. The two Rothamsted soils were

Table V. *Pot experiments on barley and mustard* 1928, 1929, 1931 (*conducted by W. E. Brenchley*).

Comparisons of increasing amounts of ammonium sulphate (S) and calcium cyanamide (Cy.) in pots with 10 kg. soil. Soils: Millstone Grit (M.G.), Leagrave (L.C.), Rothamsted Hoos Field (R.H.), Rothamsted Broadbalk (R.B.) and Woburn (W.S.). All data in grams per pot and means of four pots, in 1928 and 1931, and of six pots in 1929.

A. Barley, 1928 (sown March 9, harvested August 9–11).

Soil ...	M.G.		L.C.		R.H.		W.S.	
Fertiliser ...	S	Cy.	S	Cy.	S	Cy.	S	Cy.
Dry grain								
No N	18·9	—	18·7	—	13·6	—	12·6	—
0·106 N	22·7	22·3	21·5	22·7	17·8	17·4	16·1	17·5
0·212 N	23·8	24·4	23·0	24·4	20·9	19·4	19·9	19·0
0·424 N	23·9	25·7	25·0	25·5	25·5	24·7	26·3	24·3
Dry grain + straw								
No N	42·6	—	42·5	—	30·4	—	27·8	—
0·106 N	49·1	50·6	48·2	49·5	40·2	39·2	36·3	39·8
0·212 N	51·5	53·1	50·5	52·2	47·6	45·2	45·3	43·3
0·424 N	51·6	57·0	53·3	55·1	55·2	54·3	59·2	54·2
Nitrogen in grain								
No N	0·357	—	0·272	—	0·220	—	0·226	—
0·106 N	0·412	0·417	0·308	0·340	0·245	0·251	0·252	0·271
0·212 N	0·457	0·459	0·356	0·385	0·282	0·272	0·298	0·300
0·504 N	0·504	0·576	0·430	0·441	0·398	0·359	0·393	0·382
Nitrogen in grain + straw								
No N	0·446	—	0·345	—	0·277	—	0·276	—
0·106 N	0·517	0·525	0·413	0·448	0·304	0·311	0·315	0·339
0·212 N	0·576	0·592	0·459	0·499	0·355	0·340	0·371	0·371
0·504 N	0·668	0·763	0·567	0·586	0·498	0·453	0·500	0·482

B. Mustard, 1928 (sown August 28, harvested December 10).

Soil ...	M.G.		L.C.		R.H.		W.S.	
Fertiliser ...	S	Cy.	S	Cy.	S	Cy.	S	Cy.
Dry crop								
No N	12·0	—	7·0	—	3·2	—	10·4	—
0·106 N	13·0	14·8	9·4	9·6	4·4	5·8	13·6	15·4
0·212 N	15·7	14·9	12·0	12·2	7·4	6·3	16·6	17·0
0·504 N	18·0	18·4	16·9	16·4	11·5	9·4	21·7	20·1
Nitrogen in crop								
No N	0·417	—	0·222	—	0·077	—	0·277	—
0·106 N	0·428	0·456	0·266	0·271	0·105	0·124	0·343	0·392
0·212 N	0·523	0·505	0·315	0·322	0·162	0·158	0·468	0·463
0·504 N	0·589	0·664	0·454	0·410	0·279	0·248	0·597	0·566

C. Barley, 1929 (sown March 18, harvested August 15–16).

(In the series given in brackets the whole of the fertiliser was added in the top quarter of the pot.)

Soil ...	M.G.		R.H.		R.B.	
Fertiliser ...	S	Cy.	S	Cy.	S	Cy.
Dry grain						
No N	27·6	—	5·7	—	12·8	—
0·214 N	28·6	28·3	12·4	13·4	20·8	21·1
(0·214 top) N	(28·5)	(29·1)	—	—	(20·9)	(20·6)
0·424 N	27·7	28·5	22·0	21·1	25·2	25·2

Table V (*cont.*)

Soil ...	M.G.		R.H.		R.B.	
Fertiliser ...	S	Cy.	S	Cy.	S	Cy.
Dry grain + straw						
No N	59·7	—	13·0	—	29·8	—
0·214 N	62·0	61·3	27·9	30·0	47·2	47·5
(0·214 top) N	(61·1)	(62·5)	—	—	(47·2)	(46·2)
0·424 N	60·6	62·1	47·4	45·4	56·3	56·3
Nitrogen in grain						
No N	0·511	—	0·075	—	0·188	—
0·214 N	0·605	0·591	0·145	0·162	0·264	0·272
(0·214 top) N	(0·596)	(0·628)	—	—	(0·266)	(0·253)
0·424 N	0·643	0·686	0·283	0·269	0·379	0·377
Nitrogen in grain + straw						
No N	0·676	—	0·095	—	0·239	—
0·214 N	0·788	0·779	0·190	0·208	0·346	0·354
(0·214 top) N	(0·774)	(0·818)	—	—	(0·350)	(0·329)
0·424 N	(0·846)	0·909	0·369	0·342	0·512	0·495

D. Barley, 1931 (sown March 4–6, harvested August 6 (R.B.), 10 (W.S.), 14 (M.G.)).

Soil ...	M.G.		R.B.		W.S.	
Fertiliser ...	S	Cy.	S	Cy.	S	Cy.
Dry grain						
No N	15·9	—	17·1	—	4·0	—
0·05 N	14·6	17·0	19·4	18·8	5·8	6·1
0·10 N	19·9	19·5	22·5	22·1	7·8	7·2
0·20 N	19·8	21·6	25·5	26·1	12·5	12·1
0·30 N	20·0	20·2	29·3	28·1	17·1	17·5
0·40 N	19·8	26·0	29·9	30·0	21·6	21·0
0·80 N	18·1	26·0	29·0	32·6	26·9	26·9
Dry grain + straw						
No N	33·2	—	38·6	—	9·0	—
0·05 N	30·7	36·7	43·8	43·3	13·6	14·2
0·10 N	38·6	42·9	51·8	50·1	19·7	18·4
0·20 N	43·8	47·3	57·8	58·6	29·0	27·7
0·30 N	44·8	41·3	66·3	63·2	41·9	40·8
0·40 N	44·9	52·7	67·3	65·9	52·7	48·9
0·80 N	44·5	55·6	68·6	72·3	62·2	56·7
Nitrogen in grain						
No N	0·172	—	0·228	—	0·061	—
0·05 N	0·186	0·237	0·276	0·245	0·080	0·085
0·10 N	0·256	0·243	0·276	0·274	0·087	0·084
0·20 N	0·283	0·317	0·316	0·322	0·142	0·137
0·30 N	0·321	0·298	0·386	0·377	0·186	0·187
0·40 N	0·369	0·423	0·421	0·414	0·237	0·226
0·80 N	0·403	0·612	0·614	0·598	0·390	0·358
Nitrogen in grain + straw						
No N	0·232	—	0·292	—	0·080	—
0·05 N	0·233	0·302	0·351	0·330	0·106	0·112
0·10 N	0·342	0·320	0·354	0·354	0·123	0·131
0·20 N	0·371	0·481	0·421	0·420	0·188	0·184
0·30 N	0·428	0·424	0·513	0·506	0·260	0·254
0·40 N	0·502	0·548	0·567	0·539	0·339	0·318
0·80 N	0·589	0·813	0·863	0·798	0·546	0·470

similar in mineral composition and texture. They were prepared for pot cultures by removing the flints and adding 10 per cent. of washed sand.

The Woburn soil (W.S.) was a sandy loam from Stackyard Field, at the Woburn Experimental Farm, formed on drift over the Lower Greensand. Although the soil at Woburn is generally acid, this particular soil was alkaline. It usually responded markedly to nitrogen, but after storage during the summer of 1928 it became relatively rich in available nitrogen.

The method of these pot experiments was similar to that described by Crowther and Brenchley(5). They were conducted in rather deep earthenware pots (in which nitrification has been shown to be somewhat restricted(1)), and adequate basal dressings of phosphate and potash were always supplied. Except for one treatment in 1929, all fertilisers were uniformly distributed throughout the whole of the soil. Treatments were replicated four- or sixfold. The rates of application of fertiliser, which varied from year to year, are shown in Table V, which gives the yields and total nitrogen contents of the grain and grain + straw obtained with the different treatments.

Throughout these experiments the plants grew well; the early sown barley matured properly and in Rothamsted and Woburn soils gave large responses to added nitrogen. Except in the Millstone Grit soil, the nitrogen percentage on the dry barley grain fell to a minimum with increasing amounts of nitrogen and then began to rise. In each soil the total nitrogen content of the crop, whether barley or mustard, increased linearly with the amount of added nitrogen, giving percentage recoveries which varied between 44 and 67 per cent. in different experiments.

INFLUENCE OF CALCIUM CYANAMIDE ON YIELD AND NITROGEN RECOVERY.

To compare the action of calcium cyanamide and ammonium sulphate over such a range of experiments, instead of examining each comparison separately in terms of the standard error of the yields, it is sufficient to test whether the difference between the two fertilisers increases significantly with the amount of nitrogen given. This makes efficient use of all the pots in the experiment, except those without added nitrogen, and, further, makes it possible to compare not only the results in different years but also those on different crops.

The criterion adopted is to test whether the differences (calcium cyanamide minus ammonium sulphate) in yield or nitrogen content of the crop are proportional to the amounts of nitrogen added. Separate regression lines, passing through the origin, were fitted for each of the soils of any one experiment with identical and simultaneous treatment of several soils. All the deviations from the regression lines of that experiment were taken together to estimate the standard error of the slopes of the lines.

Table VI. *Summary of differences in yields and in percentage nitrogen recoveries between plants receiving calcium cyanamide (Cy.) and ammonium sulphate (S).*

Significant differences ($P<0{\cdot}05$) marked *.

	M.G.	L.C.	R.H.	R.B.	W.S.	Mean standard error
Difference (Cy.–S) in yield of grain + straw, gm. dry matter per gm. nitrogen added						
Barley, 1928	+11·9*	+5·4	−4·3	—	−9·3*	3·76
Mustard, 1928	+ 0·9	−0·6	−4·2	—	−1·8	2·63
Barley, 1929	+ 3·1	—	−1·7	−0·3	—	3·41
Barley, 1931	+13·0* (±4·6)	—	—	+1·5 (±2·4)	−6·8* (±0·8)	3·06
Difference (Cy.–S) in percentage recovery of added nitrogen						
Barley, 1928	+18·9*	+8·6	−9·1*	—	−2·2	4·34
Mustard, 1928	+13·1*	+4·8	−5·1	—	−3·9	5·84
Barley, 1929	+13·5*	—	−3·4	−3·9	—	3·66
Barley, 1931	+23·3* (±6·2)	—	—	−7·1* (±1·3)	−7·5* (±1·5)	3·78

All the comparisons of calcium cyanamide and ammonium sulphate in the 1928–31 series of experiments are brought together on this basis in Table VI. The results show a satisfactory consistency. In yield of total dry matter calcium cyanamide twice gave significantly higher yields than ammonium sulphate in the Millstone Grit soil, and the two insignificant results were also positive. In the eight trials on Woburn and Rothamsted soils, ammonium sulphate was significantly above calcium cyanamide twice, and above, but not significantly, five times.

In the percentage nitrogen recovery, a measure of the extent to which the nitrogen applied was utilised by the plants, there were still more consistent differences. In every trial in the Millstone Grit soil calcium cyanamide gave a significantly higher recovery than ammonium sulphate; in the Leagrave soil cyanamide was slightly but insignificantly above ammonium sulphate in each trial; whilst in the Rothamsted and Woburn soils calcium cyanamide was below ammonium sulphate in each of the eight experiments, the difference just reaching the limit for significance in three of the trials. (On account of the larger number of comparisons in 1931, and irregularities in the Millstone Grit soil through fungus attack on the plants, separate standard errors are given for each of the three soils in that year, as well as the mean for the whole experiment.)

In 1929 single dressings were also applied to the top quarter of the soil in the pots. This difference in the position of the fertilisers had no effect with sulphate of ammonia, but with calcium cyanamide it slightly increased the yield and nitrogen uptake on the Millstone Grit soil and decreased them on the Rothamsted soil. In other words, concentrating the fertiliser in the surface soil tended to magnify the usual differences.

The results of the pot experiments as a whole fall into two groups: (*a*) calcium cyanamide was more effective than ammonium sulphate in the Millstone Grit soil, and probably also in the Leagrave calcareous soil; (*b*) it was slightly but consistently poorer in the Rothamsted and Woburn soils.

This grouping of the four soils bears no obvious relation to soil texture or soil reaction.

SOIL NITRATES AND THE DEVELOPMENT OF BARLEY.

An attempt to find a cause for the differences in action of the two fertilisers in the various soils requires some knowledge of the behaviour of the added nitrogen in the soil and of its effect on the growth of the plant. As was explained in the first paper (1), the 1928 barley experiment included a number of pots parallel to the main series, which were arranged with porous tubes down the middle of each pot to allow some of the soil water to be removed for analysis at suitable intervals during the growth of the plants. The treatments studied included the soils without nitrogen and those with the heaviest dressings of calcium cyanamide and ammonium sulphate. The mustard experiment of 1928 had a similar series of pots with porous tubes. The course of development of the barley plants in 1928 and again in 1929 was followed by weekly counts of total shoot numbers, both in the main experiment and in the "porous tube" series. The leaf areas and heights of the mustard plants of 1928 were measured regularly.

A comparison of the rates of formation and death of tillers in the barley experiment of 1928 with the amounts of nitrate in the soil suggested an explanation of the essential differences in the behaviour of calcium cyanamide in the two pairs of soils.

The young barley plant absorbs nitrogen as rapidly as weather and soil conditions allow, and it forms an ever-increasing number of tillers as long as sufficient further nitrogen is forthcoming. Great meristematic activity in the young shoots requires a high nitrogen supply and serves to form a temporary reserve of nitrogen within the plant. When the nitrogen supply from the soil is exhausted, or at any rate falls below that required to maintain the growth of the large number of young tillers, the smaller ones die as the older and larger ones drain away the accumulated nitrogen. The tiller numbers pass through a maximum and then rapidly fall off. In general, a high initial tillering rate is followed by a high death rate among tillers until the numbers slowly settle down close to the final ear number.

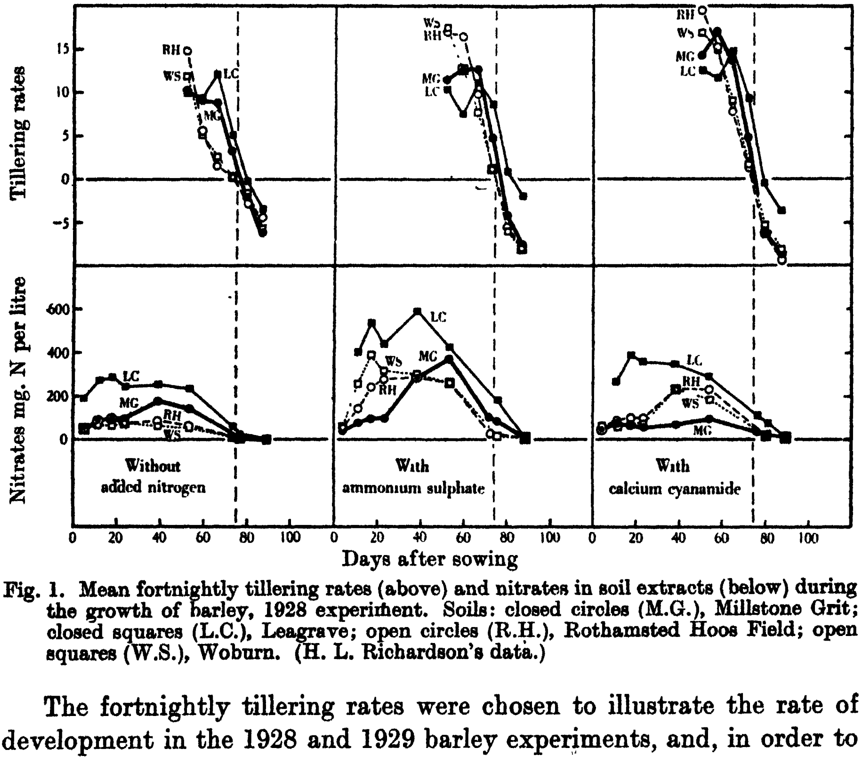

Fig. 1. Mean fortnightly tillering rates (above) and nitrates in soil extracts (below) during the growth of barley, 1928 experiment. Soils: closed circles (M.G.), Millstone Grit; closed squares (L.C.), Leagrave; open circles (R.H.), Rothamsted Hoos Field; open squares (W.S.), Woburn. (H. L. Richardson's data.)

The fortnightly tillering rates were chosen to illustrate the rate of development in the 1928 and 1929 barley experiments, and, in order to use all of the available replicates, the means for each soil in the series with added nitrogen were weighted according to the amount of nitrogen given. The fortnightly mean tillering rates per pot are plotted at weekly intervals in Fig. 1 for the four soils grouped according to treatment. On the same time scale the nitrate concentrations in similar soils with the heaviest nitrogen dressings are also given. (The individual pot results for these analyses were shown in Fig. 5 of the first paper (1).)

The tillering rate curves bring out clearly the essential differences between the four soils. In the *unmanured series* the Rothamsted and

Woburn soils gave high initial tillering rates, which fell off very rapidly —the second value plotted being but half of that one week earlier. Maximum tiller numbers (zero tillering rate) were reached 75 days after sowing, just at the time when the soil nitrates fell below the limits for analysis (less than 10 parts N per million in the soil extract). The other two soils gave somewhat lower initial tillering rates but maintained similar rates for the next two weeks, and were still tillering actively when the plants in Rothamsted and Woburn soils had ceased tillering. The Millstone Grit and Leagrave soils had much higher nitrate contents at the time of maximum tillering than the Rothamsted and Woburn soils, and still had small supplies of nitrate when those in the Rothamsted and Woburn soils were exhausted.

These relationships are shown still more clearly in the pots receiving *ammonium sulphate*. The plants in Rothamsted and Woburn soils had higher initial tillering rates and much higher rates one week later than the corresponding ones with no nitrogen, but they reached zero tillering rates at the same time as the unmanured series (75 days after sowing). The nitrates too were exhausted at this date. The ammonium sulphate did not affect early tillering rates for the Leagrave soil and only slightly increased those for the Millstone Grit soil. At 75 days after sowing, when the plants in the Rothamsted and Woburn soils had ceased tillering, those in the two richer soils were still tillering actively. The plants in the Leagrave soil at 75 days were tillering almost as rapidly as during the three preceding weeks and the nitrate content of the soil was still high—higher in fact than at any stage in the unmanured Rothamsted and Woburn soils. The Millstone Grit soil gave about half the tillering rate and also half of the nitrate content of the Leagrave soil. In both the Leagrave and the Millstone Grit soils tillers were lost more slowly (lower negative tillering rates) than in the other two soils.

These parallelisms between tillering rates near the time of maximum tiller numbers and the disappearance of nitrates appear to justify the view that, under the conditions of these experiments, changes in tiller rates may be taken to reflect changes in available nitrogen supply. Doubtless the absolute magnitude of the tillering rate depends on many other factors, both of weather and soil, but in comparisons between fertilisers supplying equal amounts of nitrogen in parallel experiments on a single soil there should be no serious error in ascribing observed differences in rate of tillering to differences in the amounts of nitrogen available to the plant.

When the action of *calcium cyanamide* is examined with this in mind, the outstanding difference between the four soils tested lies in the inhibition of nitrification—strictly, nitrate-accumulation—which was slight and of relatively short duration in the Rothamsted and Woburn soils and more marked and prolonged in the other two. The effect was so great with the Millstone Grit soil that throughout the 1927 barley experiment the nitrate concentration with calcium cyanamide was not only much below that with an equivalent amount of sulphate of ammonia, but considerably below that of the unmanured soil. Reference to Fig. 1 shows that in spite of the great reduction of nitrate concentration in the Millstone Grit soil through calcium cyanamide, the early rates of tillering were actually higher with calcium cyanamide than with ammonium sulphate. This shows that in pots treated with calcium cyanamide the nitrate content does not measure the available nitrogen.

In Table VII comparisons of calcium cyanamide and ammonium sulphate are made in terms of the mean increases in shoot numbers per pot over a three-weeks' interval in the barley experiments of 1928 and 1929. In four of the eight experiments calcium cyanamide gave significantly higher tillering rates than ammonium sulphate, and in the other four the differences, though insignificant, were in the same direction.

Table VII. *Mean increase in tiller numbers per pot from the 45th to the 66th day after sowing.*

	1928		1929	
Soil	Ammonium sulphate	Cyanamide	Ammonium sulphate	Cyanamide
Leagrave	16·0	20·5*	—	—
Millstone Grit	20·5	24·6*	21·6	26·6*
Rothamsted Hoos	25·3	25·5	27·3	28·2
Rothamsted Broadbalk	—	—	23·7	26·6*
Woburn	24·4	25·2	—	—
Standard error	0·98		0·87	

Mean of both years and all soils: S = 22·7; Cy. = 25·6*. s.e. = 0·39. Asterisks denote significant increases with calcium cyanamide.

The differences between calcium cyanamide and ammonium sulphate in their effects on soil nitrate and rates of tillering are given directly in Fig. 2 for the barley experiments of 1928 and 1929. In the Rothamsted and Woburn soils the excess nitrate accumulation from ammonium sulphate became small before active tillering started, and the slight initial superiority from calcium cyanamide in tillering rates was soon lost.

In the Leagrave and Millstone Grit soils the ammonium sulphate gave considerably more nitrate than calcium cyanamide throughout the period of active tillering. In both of these soils calcium cyanamide gave a marked superiority in initial tillering rate, lasting three or four weeks. The differences after maximum tiller numbers were somewhat erratic, though of the same general form in the two years for the two soils examined twice. A high initial rate is usually followed by a high death rate among tillers, but the actual course of the curve must depend on the relative sizes of the tillers and many other factors.

The tillering rates up to the period of maximum tiller numbers show differential effects of the same type as those in the final yields of barley.

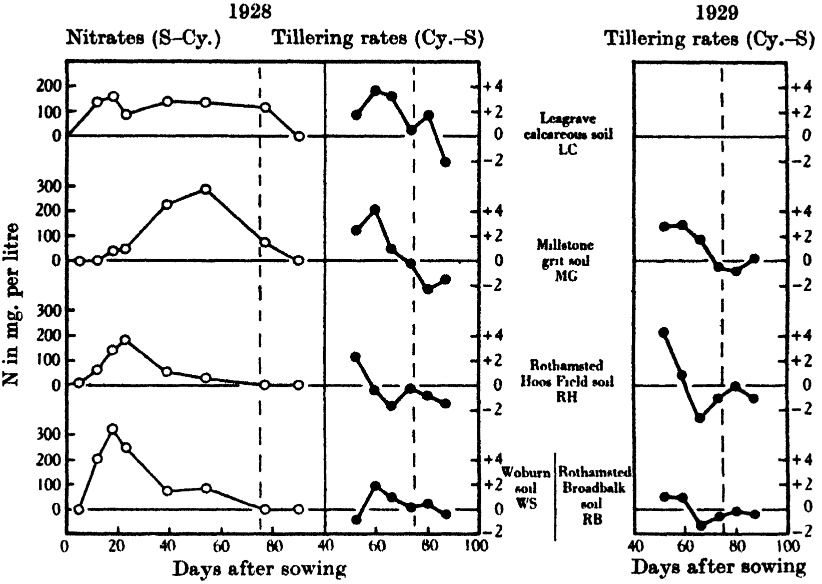

Fig. 2. Excess of nitrate in soil extract from ammonium sulphate (S) over calcium cyanamide (Cy.), in the 1928 barley experiment, and excess of fortnightly tillering rates per pot from calcium cyanamide over ammonium sulphate in the 1928 and 1929 barley experiments. (H. L. Richardson's data.)

It seems safe to attribute these differences in a large measure to the higher availability of the soil nitrogen during the period of active tillering, and to conclude that in the early stage of growth calcium cyanamide yielded more available nitrogen than ammonium sulphate in the Millstone Grit soil and probably in the Leagrave soil, but less in the Rothamsted and Woburn soils.

It was shown in the first paper, and it will be demonstrated again from other experiments that follow, that the lower nitrate content of soils with calcium cyanamide is balanced by an almost equivalent amount of ammonia. The nitrate differences (S–Cy.) in Fig. 2 may thus be regarded as measuring the extra concentration of ammonia nitrogen in the soil treated with calcium cyanamide. We advance the hypothesis that the extra tillering from calcium cyanamide in the soils in which initial nitrification is more markedly retarded, is to be ascribed to the greater availability to the plant of ammonia nitrogen as compared with nitrate nitrogen.

At the time these experiments were made this hypothesis was put forward with some diffidence, for it seemed directly counter to commonly accepted views on the relative availability of nitrogen fertilisers. There is, however, much plant physiological evidence to support it, and recent work from a number of sources, reviewed elsewhere by one of us (6), has shown that under suitable conditions ammonia nitrogen is no less available to plants than nitrate nitrogen. Indeed, in water culture experiments and in sand cultures with flowing nutrient solutions, at a pH approaching neutrality, young plants are found to absorb ammonia preferentially to nitrate, provided that the calcium and carbohydrate supplies are adequate.

In the soil the absorption of ammonium ions by colloids introduces a disturbing factor, but the results of our experiments show that the absorbed ammonia is still so readily available to plants that it may cause more rapid tillering than nitrate nitrogen. Field experiments would not necessarily show this effect as clearly as pot experiments, because of the difficulty of securing a thorough and uniform distribution of added ammonia nitrogen.

SOIL NITRATE AND AMMONIA AND THE DEVELOPMENT OF MUSTARD.

The mustard plants in the autumn experiments of 1928 developed much more rapidly than the barley in the corresponding spring experiments. The porous tube extracts showed that vigorous nitrogen uptake occurred before nitrification was complete in some of the soils. The nitrification of calcium cyanamide was again slower than that of ammonium sulphate, and unmanured pots lost their nitrate ten or more days before those with added nitrogen. The rates of growth in height roughly followed the initial nitrate contents of the soils, except that, as with barley, inhibition of nitrification by calcium cyanamide did not cause the plants to fall behind those with ammonium sulphate.

Deductions about nitrogen uptake from tillering curves and about ammonia contents from porous tube samplings of the soil water are necessarily indirect; they were therefore supplemented by experiments in which pots receiving each treatment were removed for analysis of both soil and plant at suitable stages. It is difficult to obtain adequate replication in such experiments, for apart from restrictions on the greenhouse accommodation available, the soil analyses must be made very soon after the thorough aeration to which the soil is exposed during sampling.

In an additional mustard experiment of 1928, parallel with the main series, the porous tube method of following nitrate levels was combined with periodic removal of pots for sampling the whole of the soil and the plants. The Millstone Grit soil was used, with no nitrogen and with 0·424 gm. nitrogen per 10 kg. pot as ammonium sulphate or as calcium cyanamide. The experiment began with six fold replication, which was gradually reduced as pots were removed for sampling.

Nitrate determinations were made on the porous tube extracts from individual pots at intervals of three or four days. During the early stages there was sufficient replication for the variation between pots to be measured. The percentage standard errors for a single determination, calculated on a logarithmic basis, were:

Determination no.	1–3	4–6	7–8	9–12	13–14
Days after sowing	2, 8, 12	16, 20, 23	27, 30	35, 40, 44, 48	52, 57
No. of pots per treatment	6	5	4	3	2
Standard error %	3·1	4·0	2·8	5·0	—

The uniformity of the pots and the analyses is thus satisfactory. The results of these analyses are plotted in Fig. 3 using for each treatment double lines obtained by joining points representing the mean ± 1·5 times the standard error of the mean. Except where the boundary lines for two treatments overlap, the differences in mean nitrate content for the two treatments may be taken as significant. It will be seen that over the great part of the range the curves are widely separated. Calcium cyanamide retarded nitrification for 30 days, and the nitrate concentration then rose suddenly, so that for the period from 35 to 48 days after sowing it was above that from ammonium sulphate. The general rise in nitrate concentration at 52 days coincided with the removal of the pots from an open cage into the greenhouse, where the average temperature was warmer. The results agreed well with those obtained in Millstone Grit soil in the main experiment with four soils.

Fig. 3 also gives the results of soil and plant analyses from single pots per treatment taken down at successive stages. Since single pots were concerned, isolated differences have little value, but the trend of several determinations may give useful information. The results show clearly that, in the soil, the ammonium content from calcium cyanamide remained high as long as nitrification was inhibited, but the sum of the ammonia and nitrate nitrogen in the calcium cyanamide pots was slightly below that from sulphate of ammonia. In the first four weeks, while the plants were small, those with calcium cyanamide had a slightly but consistently higher percentage of nitrogen in the dry matter than those from ammonium sulphate (Means: calcium cyanamide 6·24 per cent.; ammonium sulphate 6·05 per cent.; no added nitrogen 5·59 per cent.). The extra nitrogen appears to have encouraged growth, for, subsequently,

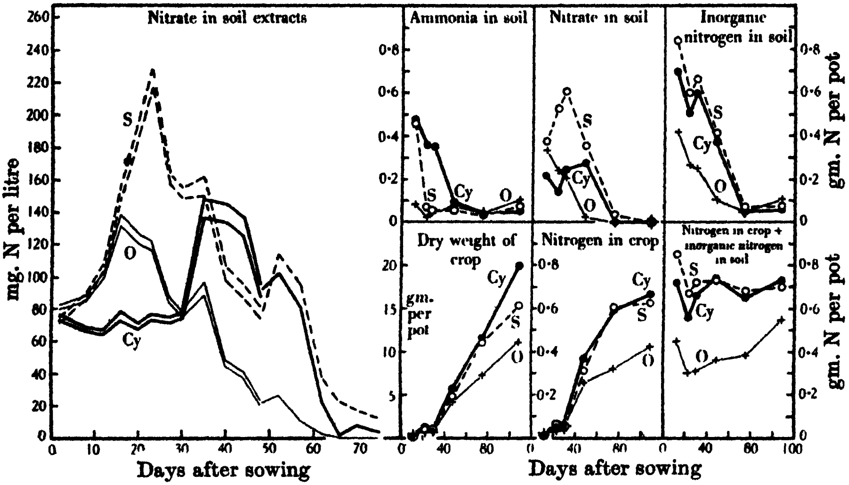

Fig. 3. Inorganic soil nitrogen and nitrogen in crop during the growth of mustard, in the 1928 experiment, with ammonium sulphate (S), calcium cyanamide (Cy.), and no added nitrogen (O). Left: nitrate in porous tube soil extracts, as mg. N per litre. Right: nitrogen in gm. per pot. as ammonia and nitrate in soil and as total nitrogen in crop. (H. L. Richardson's data.)

the plants with cyanamide had more dry matter but no higher nitrogen percentage than the ammonium sulphate plants. The leaf area was also somewhat larger with calcium cyanamide.

These results again suggest that the low initial soil nitrate content with calcium cyanamide, as compared with ammonium sulphate, is no handicap to the plant in obtaining its nitrogen, but that the low nitrate level is counterbalanced by the great availability of the ammonia remaining.

The nitrogen balance throughout this experiment illustrates, and partially explains, the low recovery of added nitrogen which is generally found in pot and field experiments. In the first three samplings almost the whole of the added ammonium sulphate (0·42 gm. nitrogen per pot) could be accounted for in the nitrogen present in the plant and in inorganic forms in the soil. Subsequently the sum of plant and inorganic soil nitrogen in the pots without added nitrogen increased steadily, whilst that for pots with nitrogenous fertilisers remained practically constant. The recovery of added nitrogen steadily decreased; in the main mustard experiment of 1928 about one-half of the added nitrogen was recovered in the final crop, although only traces remained as nitrate in the soil. Similar results have been obtained in many other pot culture experiments. When no nitrogenous fertiliser is given, there is a slow production and uptake of available nitrogen from the soil during the later stages of growth, but when a nitrogenous fertiliser is added, this extra nitrogen is not liberated.

SUMMARY.

1. In pot culture experiments with barley and mustard conducted in several soils over a number of years, the yield differences between calcium cyanamide and ammonium sulphate were generally small. Calcium cyanamide gave slightly poorer results than ammonium sulphate in soils with high responses to added nitrogen but definitely better ones in soils which contained much available nitrogen and in which calcium cyanamide greatly retarded nitrification.

2. The pot culture experiments confirmed the conclusion from earlier laboratory work that in normal soils calcium cyanamide was converted through urea into ammonia within a few days. Nitrate accumulation was less complete and slower from calcium cyanamide than from ammonium sulphate. In one soil the nitrogen from calcium cyanamide remained as ammonia for several weeks, the nitrate content being below that even of unmanured soil.

3. Where the ammonia from calcium cyanamide remained for several weeks, tillering of barley was more rapid and the final yields and nitrogen contents were higher than with ammonium sulphate. It is suggested that the young barley plant utilises ammonia nitrogen more readily than nitrate, provided the ammonia is thoroughly distributed through the soil. It is clear that the amount of nitrate obtained in nitrification tests should not be used as a measure of the relative values of calcium cyanamide and other nitrogenous fertilisers.

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(*Received August 3rd*, 1934.

THE CONTROL OF VARIATION IN GAIN IN ANIMAL NUTRITION EXPERIMENTS.

By GEORGE DUNLOP.

(Ministry of Agriculture Research Scholar.)

(From the Animal Nutrition Research Institute, School of Agriculture, University of Cambridge.)

(With One Text-figure.)

In a previous communication (1) attention was directed to the comparatively small variation obtainable in animal nutrition experiments when control was exercised over the food intake of the animals after the initial weights had been equalised. It was pointed out, however, that even under these conditions, there still existed a certain amount of individual variation which would have to be explained if the present mechanistic conception of the animal as a converter of energy was to be substantiated. It is the purpose of the present communication to present evidence that the observed individual variation, using the new feeding method, is more imaginary than real and results mainly from the false assumption that live-weight increase *per se* is a reliable measure for determining the nutritive value of rations. The results reported are somewhat scanty and if they cannot be regarded as conclusive they are at least extremely suggestive. Since circumstances do not permit the continuance of these investigations, however, their publication becomes necessary.

Besides food intake and initial weight there remain at least three other factors which govern the potentiality of live-weight increase in animals. These are (1) basal metabolism, (2) efficiency of digestion of the food, and (3) the type of energy (*i.e.* fat or protein) being laid down as live-weight increase in the body.

Basal metabolism.

To determine in how far basal metabolism affects live-weight increase two animals were selected from a group of thirty, all of which had been placed on the same plane of nutrition. One of the animals selected had shown the greatest gain, the other the least gain, in live-weight increase over a period of 7 weeks. They were placed in the animal calorimeters of this institute by kind permission of Dr Deighton to whom the writer is indebted for the basal metabolism determinations.

At a later date, when the animals were approaching bacon weight, they were again confined in the calorimeter and a second series of readings were obtained.

The relevant data are given in Table I.

Table I. *Fasting catabolism determinations.*

	Period 1		Period 2	
Animal	No. 277 lb.	No. 292 lb.	No. 277 lb.	No. 292 lb.
Initial weight	38	39	104½	105½
Weight 7 weeks later	85	78	178	177
Gain in weight	47	39	73½	71½
Weight on entering calorimeter	91	78	203	177
Weight on leaving	82	71	180	155
Faeces	5½	5¼	8½	7¼
Fasting catabolism, cals./min.	1·22	0·89	1·56	1·20
Increase in fasting catabolism between periods, cals./min.	0·34	0·31	—	—

From Table I it will be seen that animal no. 277 made the greatest gain in weight during both the first and second periods. Since both animals were fed similarly the basal heat production of no. 277 should be lower than that of no. 292 if the differences are to be accounted for by basal metabolism. The opposite, however, is the case. The data from these animals, the two extremes out of a random sample of thirty, should undoubtedly have shown some positive trend if basal metabolism exerts any marked effect on live-weight increase in swine. The actual negative result obtained with these animals showed the futility of continuing this line of enquiry further. A probable explanation of the apparent paradox will be given later.

EFFICIENCY OF THE DIGESTIVE SYSTEM.

No actual digestibility trials were undertaken with the animals. The data of Woodman (see (2) for complete list of references), obtained in digestibility trials on numbers of pigs from the same herd as those used in our investigations, show straight away that, while the experimental error of these trials is extremely small, it is greater than any difference ascribable to an actual individual variation in capacity for digestion of the foodstuffs and absorption of the food nutrients. While it is true that foodstuffs fed at different levels show corresponding differences in digestibility, no reference in the literature can be found to show that any two pigs on the same plane of nutrition have different digestive capacities for foodstuffs. During an attack of scouring digestibility may decrease, but in such cases the physiological state of the animal is abnormal. The conclusion that digestion of the food can play no important part in causing variation in the live-weight gains of our experimental animals appears justified.

ENERGY VALUE OF LIVE-WEIGHT GAIN.

The third factor to be considered offers a more probable explanation of the variation in the live-weight gain of our animals. Leathes and Raper (3) have already pointed out that carbohydrate and protein cannot be stored dry, but retain with them three or more parts of water, while fat can be stored in almost the pure state. Fat also has a greater calorific value than an equal weight of protein and carbohydrate. Retention of a gram of fat gives the body 9 calories of reserve energy, while retention of a gram of water-soaked glycogen or protein provides only about 1 calorie. These facts show that the inherited capacity of some animals to lay down more fat in a unit of live-weight gain than others should manifest itself in a lower live-weight gain. Determination of the exact proportions of the various constituents, however, would necessitate a chemical analysis of the whole carcase, which, of course, is an impracticable procedure in our experimental work. It was deemed advisable therefore to resort to the less laborious method of taking various carcase measurements indicative of the amount of fat laid down in the animals. On the accumulation of sufficient data the correlation coefficients between the carcase measurements and the growth rates of the animals were to be determined. Unfortunately, as mentioned previously, no further data will come to hand.

Recently, however, Hancock and Ellis (4) have shown that the average thickness of back fat (average of four separate measurements) gives a reliable index to the percentage of fat in the carcase. The correlation coefficient representing the relationship between these two characteristics was found to be $+0{\cdot}84 \pm 0{\cdot}04$. The measurements taken at this institute include data for the minimal depth of back fat. The number of observations obtained do not permit of a statistical study on the lines of Hancock and Ellis, but their findings, considered in conjunction with our own (*vide infra*), show clearly that minimum depth of back fat is highly correlated with the percentage of fat in the carcase and consequently, as we maintain, with rate of live-weight increase.

In the first column of Tables II and III are given the live weights of animals on the same plane of nutrition after similar periods of growth.

Column 2 shows the measurements of the minimum depth of back fat of these animals at slaughter. The data in column 3 have been obtained by multiplying the observed live weights in column 1 by the arbitrary factor $\frac{10+\text{observed back fat}}{10+\text{average back fat}}$ (the average back fat being calculated from the observed back-fat measurements of all animals receiving the same basal ration). The result is termed "corrected live weight" or lb. of live weight containing the same amounts of fat and protein. That the amount of variation has been greatly reduced, is evident from a comparison of columns 1 and 3.

Table II.

Ration 1			Ration 2			Ration 3			Ration 4		
1	2	3	1	2	3	1	2	3	1	2	3
185	2·3	182	173	2·2	169	178	3·7	186	191	1·9	174
189	2·3	186	165	2·8	169	188	3·5	193	176	3·4	180
179	2·8	183	163	3·2	172	—	—	—	—	—	—
186	2·2	182	168	2·3	165	—	—	—	—	—	—
182	2·7	185	175	2·3	171	—	—	—	—	—	—
184	2·6	185	174	2·1	168	—	—	—	—	—	—
—	—	—	170	2·3	167	—	—	—	—	—	—
Average back fat = 2·5						Average back fat = 3·1					

Column 1. Actual live weight of animals 15 weeks after reaching 50 lb.
Column 2. Back-fat measurements at slaughter (190–215 lb.) live weight.
Column 3. Corrected live weight—column 1 × $\frac{10+\text{back fat}}{10+\text{average back fat of group}}$.
Ration 1. Contains 2 per cent. $CaCO_3$.
Ration 2. Same as ration 1 with 4 per cent. $CaCO_3$.
Ration 3. 65 barley meal, 25 weatings, 10 fish meal.
Ration 4. 25 barley meal, 65 weatings, 10 fish meal.

Table III.

Ration 1			Ration 2			Ration 3			Ration 4			Ration 5		
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
202	2·0	192	187	3·1	193	194	2·4	190	176	3·0	181	196	1·9	184
—	—	—	—	—	—	181	3·0	186	174	2·9	178	175	2·6	175
—	—	—	—	—	—	183	3·0	188	195	2·0	185	173	3·0	178
Average		192			193			188			182			179

Column 1. Actual live weight of animals 13 weeks after reaching 80 lb.
Column 2. Back-fat measurements at slaughter (190–215 lb.) live weight.
Column 3. Corrected live weight—column 1 × $\frac{10+\text{back fat}}{10+\text{average back fat of group}}$.
Ration 1. Basal.
Ration 2. Basal + $\frac{1}{2}$ per cent. $CaCO_3$.
Ration 3. Basal + 1 per cent. $CaCO_3$.
Ration 4. Basal + 2 per cent. $CaCO_3$.
Ration 5. Basal + 4 per cent. $CaCO_3$.

In one batch of ten animals the back-fat measurements could not be taken. Some indication of the amount of shoulder back fat on the animals, however, was obtained from the official graders of a Pigs' Marketing Scheme at present in operation in this country. The comparative live weights of these animals, along with their grade of back fat, are given in Table IV. The data show the same trend as in Tables II and III.

Table IV.

Ration 2		Ration 3		Ration 4	
A	C	A	C	A	C
—	188	193	187	186	180
—	194	—	185	192	—
—	—	—	186	190	—

Live weights of animals 13 weeks after reaching 80 lb.
Rations as in Table III.
A. Shoulder back fat less than 5 cm.
C. Shoulder back fat greater than 5 cm.

DISCUSSION.

The cause of the difference in live-weight gain of the two animals selected for our basal metabolism studies during period 1 is now clear. The slower growing animal (no. 292) had laid down a greater proportion of fat to protein than the other. This was confirmed at slaughter when the back-fat measurement of animal no. 277 was found to be 2·9 cm. and that of animal no. 292, 3·1 cm. These findings also explain the higher basal metabolism per unit weight in animal no. 277. With a smaller proportion of fat to protein in its live weight it naturally had the greater protoplasmic mass to which basal metabolism is known to be related.

During period 2 the live-weight increase of the two animals showed little difference, which suggests that fat and protein were being laid down in similar amounts. The increase in the basal metabolism of the two animals during this period proves that such is the case, since the respective increases over the determinations at the end of the first period were 0·34 and 0·31 cals./min. respectively. Had one of the animals shown a lower gain during the second period we should have expected its increase in basal metabolism to be less owing to the greater amount of fat to protein in its gain.

The results in general indicate that a much greater degree of precision is possible in nutrition experiments conducted either according to the paired feeding method, or to the method evolved at this institute, when variability in the energy content of live-weight gain is taken into consideration and suitably corrected for. Clausen (7) has shown that the amount of protein or muscle laid down by the animal is an inheritable characteristic, and is consequently beyond the control of the nutrition worker in his experiments. The amount of energy left over from the food, after the animals' requirement for protein has been met, will be laid down as fat. Thus, in animals fed according to the new technique, the amount of fat deposited will depend on the inherent capacity of the animal to lay down protein, since the food intake is controlled. Some simple measurements enable us to determine fairly accurately the fat percentage of the live-weight gain of the animals, and by correcting to standardise the gains on an equal fat content basis, a more exact measurement of the nutritive value of the ration is obtained.

It is probable that the minimal back-fat measurement chosen for live-weight correction purposes is not the most suitable. The average back-fat measurement as obtained by Hancock and Ellis (4) would appear to give the best index of the percentage of fat in the carcase. On using their measurement for live weight correction purposes, the variation in the corrected live weights would be further reduced, and it is apparent that the experimental error must be about the same magnitude as in analyses of foodstuffs by chemical methods. On first principles, the technique suggests itself as a more suitable and desirable method of determining the feeding value of animal foods, rather than laborious digestion trials in which the chemical methods for estimation of the digestible food nutrients are still suspect (5).

In conclusion we should like to draw attention to the importance of controlling food consumption so that each experimental animal assumes the same plane of nutrition. Morris, Palmer and Kennedy (6) found that when animals fed *ad lib.* had consumed the same amounts of food over a period of time, they, nevertheless, made widely divergent gains in weight. They concluded that the correlation coefficient between dry matter consumed and gain in weight is not high enough to warrant the use of the paired-feeding method with small numbers of animals. Similarly, Yates (8), using the individual *ad lib.* feeding technique, found that there was little reduction of variability when he adjusted the final weights of his animals for differences in food consumption by the analyses of covariance. He ascribes the lower variability we obtained by the technique adopted at this institute to the greater homogeneity of the material and the better experimental conditions at Cambridge.

The findings of these workers are interesting in view of the consistently low variability which has been found wherever the paired feeding or the new method has been adopted. It is unlikely that the homogeneity of the material, as far as food utilisation goes, is greater at these centres than in the stocks and herds at Minnesota and Rothamsted respectively. We must therefore postulate, as Yates tentatively suggests, that their technique exercises no control over other factors which make for higher error.

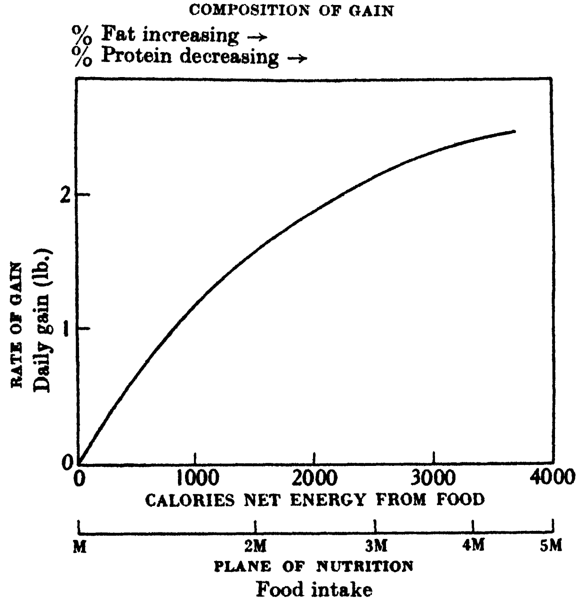

Fig. 1. The figure is only roughly quantitative. Along the abscissae are plotted the calories of net energy available from the food. The supplementary scale shows that the net energy from each succeeding unit of food intake becomes less as the plane of nutrition is increased above maintenance (*M*) (see Brody(9)). The figure illustrates that the relationship between net energy and live-weight gain is not linear. As the daily gain (measured in lb.) increases, the greater is the expenditure of net energy (as calories) for each succeeding increment of gain. This is due to the increasing proportions of fat to protein laid down as live weight when the daily gain increases.

One such factor is clearly evident from a consideration of the technique adopted in the individual *ad lib.* feeding method and that adopted in the paired feeding and Cambridge methods. For while it is true that the above workers have corrected for varying food consumption, they have nevertheless left uncontrolled the apparently much more important factor, namely, plane of nutrition. Although their animals consumed equal amounts of food during the experimental period, they need not necessarily have been on the same plane of nutrition. Indeed, their consecutive daily food intakes may have varied enormously. It is also possible that one animal would consume a greater amount of food during the first half of the period and less during the second half than its "pair" mate, *although both consumed the same amount during the entire period.* In view of Brody's findings, regarding the data of Forbes and Mitchell (9), and the ideas expressed in Table I it is only natural that these animals should make different utilisation of their food for the purposes of live-weight increase. This factor alone probably accounts for the large differences in gain of the "pair" individuals of Morris *et al.* compared with the differences obtained by Mitchell ((6), p. 41), who exercises rigorous control over the food intake of his animals.

It is possible, of course, that the differences in gains obtained by Morris *et al.* might be due to difference in the composition of gains (proportions of fat to protein) which has been shown earlier (p. 154) to account largely for the residual variation in the Cambridge technique. We believe, however, that their differences are too great to be accounted for in this way. Besides, Mitchell's figures show the increased precision obtainable when the daily food intake is controlled, and it appears more logical to attribute the differences of Morris *et al.* to failure to control this factor. The comparatively small amount of variation which remains after this is accomplished (cf. Mitchell's data (6)) can then be largely eliminated by equalising the energy content of the live-weight gains by some method similar to our own.

SUMMARY.

The cause of variation in the live-weight increase of animals on experiment has been further investigated. It had been shown previously that varying food intakes and initial weights—inherent drawbacks of the group-feeding method—accounted for much of this variation. When these two factors are rigidly controlled small differences in live-weight gains are still obtained. Evidence is presented to show that the basal metabolism and the digestive capacity of animals are for all purposes the same, and cannot account for any great part of the residual variation.

Our investigations indicate that differences in the proportion of fat to protein laid down affect the magnitude of the gains of the animals. It has been found that measurement of thickness of back fat is a reliable index to percentage of fat in the carcase, and consequently to the proportion of fat to protein laid down. By correcting the observed live weights by a factor incorporating these back-fat measurements, the gains can be calculated on an equal energy basis. These "corrected live weights" show little variation among individuals on one treatment (standard deviation approximately 1 per cent.).

The advisability of obtaining the back-fat measurements according to the method of Hancock and Ellis is discussed.

It appears that equalisation of the food intake over the experimental period allows for comparatively large variations in live-weight gains. The necessity of keeping the plane of nutrition constant either in accordance with increasing live weight (as in our technique) or from day to day (as in the paired-feeding trial technique) is stressed.

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(*Received October* 1*st*, 1934.)

VOLUME XXV APRIL, 1935 PART 2

FACTORS INFLUENCING SEED-SETTING IN ALFALFA[1].

BY J. M. ARMSTRONG[2] AND W. J. WHITE[3].

(Division of Agrostology, Central Experimental Farm,
Ottawa, Canada.)

(With Plate I and Five Text-figures.)

THE improvement of alfalfa with respect to seed production is one of the major projects of the Dominion Department of Agriculture. The problem is being attacked chiefly from the standpoint of genetics, but some attention is being given also to various other factors which influence seed-setting. The basis of this study is a special group of plants which produce seed abundantly and normally by self-fertilisation. These have been described by Kirk and White (1933), and their possible utility in breeding for seed production has been fully discussed by Kirk (1933).

A natural development in breeding work with these autogamous alfalfa plants was the obvious necessity of determining the mechanism by which pollination and fertilisation are effected in the absence of any external agency responsible for tripping of the flowers. It was desired to know precisely why a high percentage of the flowers of these plants consistently produced pods when protected from insect pollinators, whereas the generality of alfalfa plants produce little or no seed unless the flowers are artificially manipulated. A general study was undertaken, therefore, of various factors which might have a bearing on pollination and fertilisation in the case both of ordinary alfalfa and the normally self-fertilised plants. These latter provided material which had not been available to investigators who had previously studied this subject, and it was felt that perhaps new information might be obtained which would help to elucidate some of the conflicting results which have hitherto been reported in numerous papers on this subject.

In presenting the results of our investigations, the various factors which were studied will be discussed in order, beginning with a consideration of the structure of the alfalfa flower. References to the literature will be cited under each heading in connection with the matter under discussion.

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[1] Contribution from the Division of Forage Plants, Dominion Experimental Farms, Ottawa, Canada.

[2] Research Assistant, Division of Forage Plants, Ottawa.

[3] Research Assistant, Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan.

The experimental work reported in the present study has been carried on entirely in greenhouses during the past two years. The plants have been classified into two groups on the basis of their seed-setting capacity under greenhouse conditions, high seed-setting types or autogamous plants, and low seed-setting types consisting of random plants from Grimm strains.

The greenhouses were screened to exclude insects, and all the common greenhouse insect pests were kept under control. The plants were grown in 4 in. and 5 in. pots and repotted in fresh earth every time a seed crop was harvested. They were watered either on the surface or by flooding the benches. In the winter the short hours of daylight were supplemented by the use of artificial light. Pod-setting data were obtained by counting the total flowers on a group of tagged racemes followed later by a count of the number of pods set, or by the equally reliable and time-saving method of determining the number of flowers per raceme by counting the pods and empty stipules from which the unfertilised flowers had fallen.

The most important phases of the work were duplicated as far as possible at the Dominion Forage Crops Laboratory at Saskatoon, and at the Central Experimental Farm, Ottawa. In addition, certain particular phases of the problem were carried out independently by each author.

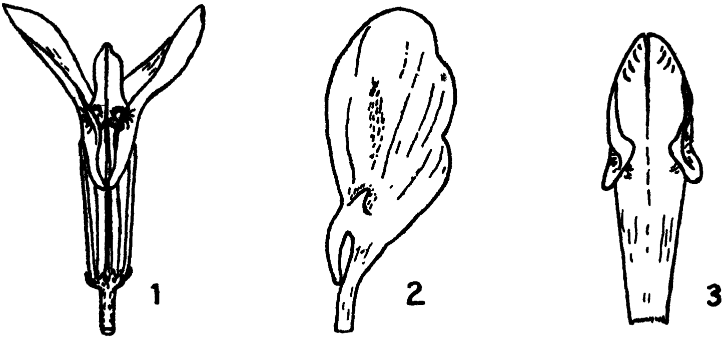
STRUCTURE OF THE ALFALFA FLOWER.

The most interesting feature of the alfalfa flower is the tripping or exploding mechanism. In the untripped flower the staminal column is enclosed under tension within the keel. On the keel there are two processes, in the nature of evaginations, which meet in a slight groove in front of the staminal column and prevent it from springing forward. This restraining force is further augmented by a horn-like projection on the side of each wing which fits into the keel evaginations (Text-figs. 1–3).

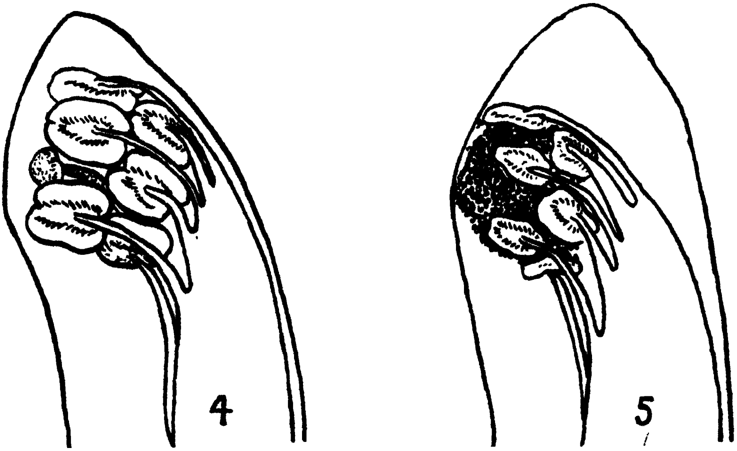
Tripping is accomplished by any force which tends to separate the front edges of the keel. This opening of the keel withdraws the keel processes from their position in front of the staminal column which immediately springs forward until it strikes the standard. The upper part of the standard is soft and flexible, while the lower half is composed of firmer and more rigid tissue. The staminal column strikes the rigid area;

consequently the standard yields little under the relatively strong impact of the column.

The upper portion of the style has several important morphological adaptations to ensure fertilisation. It is strongly curved so that the



Text-figs. 1–3. Alfalfa flower parts drawn with the aid of a camera lucida. Text-fig. 1. Front view of a flower with calyx and standard removed, showing the position of wing and keel processes. Text-fig. 2. Interior surface of a wing petal. Text-fig. 3. Front view of the keel petals.



Text-figs. 4–5. Normal conditions within the keel at the straight bud and erect standard stages respectively. The anthers surround the stigma in a double ring. In Text-fig. 5 anther dehiscence is finished and the anther sacs are somewhat shrunken; the pollen completely covers the stigma.

maximum amount of stigmatic surface comes in contact with the standard. In cross-section it is cuneiform and possesses a cortex of thick-walled cells to a depth of from two to four layers (Plate I, fig. 5). These features prevent the pistil from buckling under the force of the impact and are especially necessary since the staminal column redivides into its constituent stamens near the top, thus depriving this portion of the style of any support.

The flower, during the course of its development from the small bud to the open flower, passes through four fairly definite stages. These Coffman (1922) has carefully described and termed the straight bud, the pointed bud, the hooded bud and the erect standard stages. These definitions have been accepted for use in the present study.

RELATION OF POLLINATION TO SEED-SETTING.

The fact that alfalfa pollen is shed in the bud stages and is present on the stigma was discovered by the early investigators, Hildebrand (1866), Muller (1873), and Burkill (1894). In recent years this discovery has been confirmed by Roberts (1916), Coffman (1922), Engelbert (1932), and Kirk and White (1933). Several workers, Brand and Westgate (1909), Piper *et al.* (1914), and Helmbold (1929), have expressed the view that pollination is *not effective* until the flowers are tripped.

In the present study, pollination of alfalfa flowers was investigated with respect to (1) the stage of dehiscence of the anthers, (2) the amount of the pollen, (3) the distribution of pollen within the keel of unopened flowers, and (4) the relative positions of stigma and anthers. Observations with respect to each of these factors are recorded for individual plants of both the high seed-setting and low seed-setting types. It was recognised that if these should differ conspicuously in any one of the above-mentioned particulars, the information obtained would assist materially in identifying factors conditioning autogamous seed production.

To determine the stage of dehiscence of the anthers several blossoms at the four stages of development were examined from each plant, using the technique previously published by Kirk and White (1933).

Table I. *Stages of anther dehiscence in alfalfa plants of the high seed-setting and low seed-setting types.*

		Stages of anther dehiscence				
Plant type	Place	Straight bud	Pointed bud	Hooded bud	Erect standard	Total
High seed-setters	Saskatoon	1	10	1	—	12
	Ottawa	1	10	—	—	11
Low seed-setters	Saskatoon	1	11	2	—	14
	Ottawa	2	15	3	—	20
Miscellaneous	Saskatoon	3	22	3	—	28
	Ottawa	2	12	—	—	14
Total number of plants examined		10	80	9	—	99

It is seen from Table I that 80 of the 99 plants examined shed their pollen in the pointed bud stage; in 10 plants dehiscence had started in the straight bud stage and in 9 it was delayed until the hooded bud stage. The high seed-setting types do not differ from the low seed-setting types with regard to the stage of dehiscence. Observations on a number of miscellaneous plants belonging to five distinct varieties are also included in the table. Their behaviour differed in no way from that of the main experimental material and indicates that anther dehiscence in the pointed bud stage is the general rule.

When pollen was found to be shed in the straight bud stage the quantity was very small. Even in the pointed bud stage the anthers were not completely dehisced, dehiscence being a gradual process. In the erect standard stage it was observed in most cases that some or all of the anthers were completely dehisced and the anther sacs withered, indicating that dehiscence is regularly completed previous to tripping.

Differences were observed in the total quantity of dehisced pollen at the erect standard stage as follows:

	Abundant	Medium	Scarce
High seed-setters	23	—	—
Low seed-setters	29	4	1
Miscellaneous	37	3	2

In all of the high seed-setting types there was an abundant quantity of dehisced pollen. This cannot be regarded, however, as a distinctive characteristic, since most of the checks also possessed abundant dehisced pollen. It may be considered, however, a necessary prerequisite to high seed production. Helmbold (1929), Torssell (1929), and Engelbert (1932) have also observed that individual plants differ in regard to the abundance of pollen and emphasise the importance of selecting good pollen-producing plants as breeding material.

Observations on the distribution of pollen within the keel showed that in all cases, except where the amount of dehisced pollen was scarce, the pollen was found to be on and around the stigma at the erect standard stage. Pollen frequently filled the small boat-shaped space between the anthers and the tip of the keel. In the straight bud stage the anthers were closely imbricated and entirely surrounded the stigma in a double row, the filaments being alternately long and short (Text-fig. 4). At this stage the stigma was seen to protrude slightly above the anthers. The dehiscence of the pollen occurred only on the inner surface of the anthers, and as dehiscence proceeded the pollen was forced inward and upward until finally the stigma was completely covered (Text-fig. 5).

No significant differences were observed between the high seed producers and the checks with regard to the relative position of stigma and anthers, such as Kirk and Stevenson (1931) observed in sweet clover. The pistil was consistently slightly longer than the surrounding anthers. This slight difference did not inhibit self-pollination except in cases where the amount of dehisced pollen was scarce.

This phase of the study has shown that the high seed-producing selections do not differ from the population from which they were selected in any factors affecting self-pollination. Both types shed their pollen regularly in the bud, and when the erect standard stage has been reached the pollen is sufficiently abundant to bring about self-pollination.

AUTOMATIC TRIPPING IN RELATION TO POD-SETTING.

Several investigators, particularly in recent years, have reported that pod-setting occurs even when the flowers have not been tripped. Urban (1873) and Piper *et al.* (1914) found that in rare cases untripped flowers formed pods. Carlson (1930), and Carlson and Stewart (1931), were the first workers to report any material setting of pods without tripping. In one experiment, under field conditions, Carlson estimated that 26 per cent. of the flowers produced pods without tripping. Kirk and White (1933), as a result of a preliminary examination of our present research material, considered that tripping of the flowers probably was not necessary for fertilisation. Burkill (1894), Hackbarth (1930) and Ufer (1932), on the other hand, state without reserve that fertilisation only occurs if the flowers are tripped.

Considerable difference of opinion therefore still exists as to the occurrence of seed-setting without tripping. It is apparent that if fertilisation without tripping does occur, this might explain the differential seed-producing capacities of high seed-setting and of low seed-setting types.

Table II. *The relation of tripping to pod-setting in the undisturbed flowers of high pod-setting and low pod-setting plants.*

Plant type	Place	No. of plants	Total no. of flowers observed	Flowers automatically tripped		Pods set	
				Total no.	%	Total no.	%
High pod-setters	Saskatoon	11	396	301	76·0	203	51·3
	Ottawa	9	375	290	77·3	212	56·5
Low pod-setters	Saskatoon	14	460	66	14·3	22	4·8
	Ottawa	23	398	62	15·6	34	8·5

Table II gives a summary of the observations in regard to the proportion of flowers which were automatically tripped and those which set pods. These data were obtained by tagging several racemes on each plant when the flowers were in the late pointed bud to early erect standard stages. These racemes were kept under observation from day to day until all of the flowers had either dropped or formed pods. The date of tripping, flower wilting, flower dropping or appearance of the pod was recorded for each flower.

High seed-setting and low seed-setting types differed markedly in the degree of self-tripping. The former were highly self-tripping under the ordinary greenhouse conditions. The two types also differed in the proportion of tripped flowers which set pods, the high seed-setters excelling in this respect.

It is seen from the table that the number of flowers automatically tripped exceeded the number of pods. Of all the racemes examined, in no case did the number of pods exceed the number of self-tripped flowers. White, at Saskatoon, obtained a correlation of 0·90 ± 0·01 between tripping and pod-setting. This high positive correlation, together with the above observations, prove beyond doubt that automatic tripping was the principal factor determining the amount of pod-setting.

Two main conclusions may be drawn from this phase of the investigation. Firstly, that self-tripping is the factor which very largely determines the differential seed-setting capacity of alfalfa plants. Secondly, that fertilisation apparently does not occur in untripped flowers in spite of the fact, previously demonstrated, that pollination has taken place.

FACTORS AFFECTING AUTOMATIC TRIPPING.

That alfalfa flowers sometimes trip automatically is by no means a new observation. Piper *et al.* (1914) state that its occurrence was first actually observed at Chinook, Montana, in 1909. These authors, and also Blinn (1920), observed that automatic tripping takes place most frequently in the hot sunshine. Piper found it could be brought about by focusing a burning glass on the open flowers or by simply bringing shaded flowers into the sunshine on a hot day. Dwyer (1932) carried out laboratory tests to determine the effect of temperature on tripping. He found that some tripping occurred at 101° F. and that instantaneous tripping of all the flowers was obtained at 104° F. He found that tripping occurred at this temperature under the most diverse moisture conditions, provided the flowers were in a fresh turgid condition.

The aim of the present study was to discover any morphological differences in the flowers of the two seed-setting types which might affect the relative ease of tripping. Certain environmental effects which were noted incidentally and which applied equally to both types will be dealt with first.

Flowers were observed to trip more freely in the spring than in mid-winter. The duration and intensity of the hours of sunshine appeared to be the controlling factor. The use of artificial light in the winter months did not appear to make up for deficiency of sunlight so far as tripping was concerned, although it was very effective in inducing profuse flowering. While greenhouse temperatures were somewhat higher in spring than in winter they never approached that which Dwyer found was required for automatic tripping. Automatic tripping and pod-setting were also found to be greatly lessened if there was a depletion of the soil nutrient supply. Light spraying of the low seed-setting types was found to be effective in causing increased tripping with, however, no increase in pod-setting.

An examination of the tripping mechanism of high and low seed-setters showed striking morphological differences. These consisted of (1) differences in the length of the anterior wing processes and their degree of adherence to the keel, and (2) differences in the size and tension of the staminal column. The mean length (based on 15 flowers from each plant) of the anterior wing processes in one high seed-setting plant was 3·64 ± 0·075 units, and in a low seed-setter 4·32 ± 0·109 units, a difference which is statistically significant. The length of the wing processes varied with the depth of the keel invaginations, and the less pronounced keel processes permitted the tripping mechanism to be released more easily. More marked even than differences in the restraining mechanism were differences in the vigour with which the staminal column tripped. Weak keel processes, however, were not always associated with high-tension staminal columns in the high seed-setters. It would appear that the ease of tripping is determined by a balance between the dynamic force exerted by the staminal column and the static force present in the keel and wings.

It may be concluded from the above observations that at least two of the factors which determine autogamous seed production are evident in the morphology of the tripping mechanism, and also that these factors are fairly sensitive to environmental conditions.

GERMINATION OF THE POLLEN ON THE STIGMAS OF ALFALFA FLOWERS AND GROWTH OF THE POLLEN TUBES.

In view of the fact that pollen is deposited on the stigmas of both tripped and untripped flowers and that untripped flowers invariably drop off while a large percentage of tripped flowers set pods, a study of the germination of the pollen on the stigmas of tripped and untripped flowers was undertaken.

Martin (1915) studied the germination of alfalfa pollen in relation to moisture requirements. He found that pollen germination depends upon the moisture supply which may be obtained from either the air or a moist substratum. Working with tripped flowers, slightly wilted, he found that the pollen which was massed on the anthers and standard germinated in 100 per cent. atmospheric moisture while no germination of pollen on the stigma occurred. At 90 per cent. atmospheric moisture there was much germination on the stigma but none elsewhere. He concluded that pollen is very exacting as regards moisture conditions necessary for germination, requiring a correct balance between evaporation of moisture from the stigmatic surface and its absorption by the pollen grains. Martin did not investigate the question of fertilisation in untripped flowers.

In the present study the flowers of fresh racemes were artificially tripped and left on the plants for 6–8 hours. The tripped flowers and untripped flowers, which were used as checks, were then taken to the laboratory where the flowers were dissected and the pistils mounted in a staining solution of lacto-phenol containing an acid fuchsin light green stain. The stain was made up of 8 parts of 1 per cent. aqueous acid fuchsin and 2 parts of 1 per cent. light green in 95 per cent. alcohol. Sufficient stain was added to the lacto-phenol to give it a deep colour. A cover-slip was placed over the pistils and another slide placed over the cover-slip. Vertical pressure was applied on the slide in order to flatten the pistil so that the germ tubes could be traced through the stigma. Due to the thick cortex of the style it was not possible to follow the tubes further. The pollen tubes and grains were quite well stained in 3–4 hours, and the unsealed slides could be kept for several weeks. The results obtained are summarised in Table III.

A survey of the table shows that of 253 pistils examined from tripped flowers, 213, or 84 per cent., showed pollen germination; of the 314 pistils from untripped flowers only 2, or 0·6 per cent., showed pollen germination. It is possible that one or both of these two flowers have been erroneously recorded as untripped, but assuming the record is correct, the percentage of fertilisation in untripped flowers is quite negligible. In the tripped flowers of both the high and low seed-setters there was a certain percentage which did not show any pollen germination. This is in agreement with the pod-setting data presented in Table II, where a certain proportion of the tripped flowers failed to set pods. The discrepancy from the expected pod-setting and pollen germination percentages is greater for the low seed-setters than for the high seed-setters. But for both types this discrepancy is greater for pod-setting than for pollen germination. It therefore appears that in some cases the pollen tubes never reach the ovary due to inhibition or retardation of their growth in the style, or that after fertilisation is effected the ovules abort. Martin (1915) suggests that flower dropping may be due to ovule abortion because of the plant's inability to furnish the proper water and food supply.

Table III. *Pollen germination and pollen tube growth in the stigmas of high seed-setting and low seed-setting types of alfalfa plants.*

			Tripped flowers		Untripped flowers	
Plant type	Place	No. of plants	Pollen tubes present	Pollen tubes absent	Pollen tubes present	Pollen tubes absent
High seed-setting plants	Saskatoon	7	36	4	2	201
	Ottawa	5	66	6	0	35
Low seed-setting plants	Saskatoon	5	55	5	0	10
	Ottawa	4	56	25	0	66
Total		21	213	40	2	312

The results of germination studies reported here fully explain the results obtained in the pod-setting studies. No pods set in untripped flowers because there was no germination of the pollen and hence no fertilisation.

IS THE RUPTURING OF THE STIGMA ESSENTIAL TO ITS BECOMING RECEPTIVE?

The results thus far emphasise very clearly the role and importance of tripping in seed production, and yet they fall short of answering the above question. It will be readily admitted that tripping has in some way initiated pollen germination and rendered the stigma receptive. It is conceivable, however, that this receptiveness may be brought about otherwise than through rupturing of the stigmatic surface.

Burkill (1894) stated that "the stigma does not become receptive until rubbed or until its cells are injured in some manner." The experiments of Piper *et al.* (1914) did not support this conclusion. Hackbarth

(1930) states that the papillae of the stigma are covered by a thin membrane which has to be ruptured in order to permit pollen germination.

In investigating this matter we adopted two distinct methods of procedure. At Ottawa the method of direct microscopic observation was used, while at Saskatoon a series of experiments involving different modes of stigma scarification were carried out. In the first method the pistils of freshly tripped and untripped flowers were dissected and mounted in an excess of the same staining fluid used in the germination tests. The cover-slips were laid on loosely. Microscopic observation was made with a 10 × objective, which allowed plenty of clearance above the slide, and the 20 × oculars. A very striking difference was observed between stigmas from tripped and untripped flowers (Pl. I, figs. 1–4).

Pistils from untripped flowers were found to have oval-shaped stigmas with the basal fringe of papillae closely appressed to the surface. The stain used was incapable of penetrating the stigma even after two days. The ungerminated pollen grains in every case lay so loosely on the stigma that they easily floated away. Seen in profile, the margin of the stigma was very even and clear-cut, and a thin hyaline membrane could be seen. In one case (Pl. I, fig. 4) there was a slight rupture of this membrane in mounting the pistil and the stigmatic content was observed to have partially escaped.

In the case of pistils from tripped flowers the stigmas were wider and flatter with the papillae spread out at the base. There was always strong and rapid penetration by the stain. The profile of the stigma was very irregular and the membrane was ruptured in several places. The pollen grains were embedded in the stigmatic content which had oozed out. These characteristics were observable in freshly tripped stigmas. If the pistils were mounted 5–6 hours after the occurrence of tripping, many of the pollen grains had already germinated and the tubes had penetrated into the ruptured areas of the stigmatic surface.

The work of Martin (1915) suggested another experiment. Racemes of untripped flowers were suspended by wire in corked flasks containing some water at a temperature of 80° F. for 8–10 hours. By such treatment 100 per cent. atmospheric humidity was obtained. The flowers were then dissected and the unruptured pistils mounted as before. About 20 per cent. of the pollen was observed to have germinated, but in no case was there any penetration by the pollen tubes into the stigmas.

Further evidence that pollen germination at normal greenhouse humidity can only occur when the stigmatic surface is ruptured, and also that this rupturing is normally brought about when the stigma strikes the standard, was obtained from a series of experiments performed by the second author at Saskatoon.

The flowers on from three to six racemes on each of the high pod-setting and of the low pod-setting plants, were tripped by a lateral pull on one of the wings with a pair of tweezers. This permitted the staminal column to strike only the standard. In another set of racemes the standards were removed by clipping and the flowers were then tripped. In this case the stigma did not come in contact with the standard. This experiment was performed in 1932 and duplicated in 1933. The pod-setting resulting from these two treatments is presented in Table IV.

Table IV. *Pod-setting when alfalfa flowers are mechanically tripped, with and without removal of the standard.*

		Standards previously removed by clipping			Standards undisturbed		
Plant type	No. of plants	No. of flowers treated	No. of pods set	% pod-setting	No. of flowers treated	No. of pods set	% pod-setting
High pod-setters	11	273	31	11·4	366	209	57·1
Low pod-setters	14	353	28	7·8	471	135	28·7

When the standards are clipped before tripping it is seen from the table that pod-setting is very much reduced in both types in comparison with the controls. The high pod-setters gave an average of 11·4 per cent. in comparison with 57·1 per cent. for their controls. The low pod-setters averaged 7·8 per cent. with the controls giving 28·7 per cent. To summarise the data for all plants observed during two years, 59 pods out of a possible 626 or 9·5 per cent. were obtained when the standards were removed before tripping in comparison with 344 pods out of a total of 837, or 40·8 per cent. when the stigma was allowed to strike the standard. This highly significant difference emphasises the real function of tripping and certainly indicates that the standard performs a definite role in fertilisation.

In a similar experiment, Burkill (1894) clipped the standards from 50 flowers before tripping them. Out of these none set seed, while out of 34 control flowers 12 set seed. Piper *et al.* (1914) from a similar experiment obtained no significant difference, and concluded that the mechanical effect of the stigma striking the standard is not necessary to ensure fertilisation.

From a microscopic examination of stigmas of tripped and untripped flowers, it was found that the stigmatic surface was remarkably easy to rupture, the slightest pressure on the cover-slip being sufficient. The clipping away of the upper part of the standard therefore cannot be regarded as a perfect insurance against stigmatic rupturing. There are the possibilities of the stigma striking other flowers on the same raceme or of a slight friction between the stigma and the edge of the keel or even a slight jar as the tripped column comes to rest. These factors may account for the fact that tripped flowers with standards removed did produce some few pods.

In a further experiment two methods of scarification were tried on the stigmas of tripped flowers from which the standards had been removed.

Table V. *Effect of scarification of the stigma of alfalfa flowers by different mechanical methods.*

Methods of scarification	Plant type	No. of plants	No. of flowers	No. of pods	% pod-setting
With a camel's hair brush	High seed-setters	11	273	79	28·9
	Low seed-setters	14	332	112	33·7
With a toothpick	High seed-setters	11	287	102	35·5
	Low seed-setters	14	346	66	19·1
Undisturbed	High seed-setters	11	273	31	11·4
	Low seed-setters	14	353	28	7·8

The data obtained (Table V) show that both forms of scarification brought about a marked increase of pod-setting and confirm the conclusions stated above that some form of stigma scarification is necessary in order to ensure a maximum production of seed pods. This is normally brought about when the stigma strikes the standard. This natural means of scarification appears to be more effective than the artificial methods, probably because the contact of the standard and the stigma maintains a moisture condition which provides a better medium for pollen germination.

POLLEN STERILITY AND ITS EFFECT ON SEED-SETTING.

As previously shown, there exists a considerable gap between the proportion of flowers which are tripped and those which set pods. In the hope of finding a reason for this deficiency in the number of pods produced, studies were carried out at Ottawa on the viability of the pollen.

Table VI gives the results obtained from a group of 8 hybrid plants from autogamous parents in which a very marked difference existed between flower-tripping and pod-setting. The average percentage of tripped flowers of those plants, which rate as low pod-setters, is as high

Table VI. *Pollen sterility in relation to pod-setting in tripped alfalfa flowers.*

Plant no.	Flowers automatically tripped		% of tripped flowers setting pods	% of good pollen
	Number	%		
1	13	43·3	46·2	49·7
2	35	97·2	8·6	71·6
3	27	93·1	33·3	72·6
4	15	60·0	40·0	80·0
5	29	87·9	31·0	55·5
6	14	48·3	50·0	72·5
7	24	77·4	25·0	76·6
8	34	100·0	5·9	34·5

as that of the high pod-setters given in Table II. In only 3 of the 8 plants, however (1, 5 and 8), could pollen sterility be suspected as the reason for poor pod-setting; with respect to the other plants which had good pollen in excess of 70 per cent., other factors must be responsible.

Table VII. *Relation of pollen sterility to pod-setting in a random group of first generation selfed alfalfa plants.*

Plant no.	Mean percentage of good pollen	S.E. of mean	% pod-setting
84– 3	49	±1·8	0
–11	83	±0·7	80
–65	68	±2·1	17
–74	78	±1·6	37
–78	51	±1·7	0
–85	70	±1·3	17
–88	85	±1·9	46
–89	67	±1·6	20

A random group of first generation selfed plants from a moderately high pod-setter were selected for a further analysis of pollen sterility. From each plant 10 flowers were selected for examination and the mean percentage of good pollen determined. It is seen from Table VII that there is a definite segregation of a factor or factors for pollen sterility in the plants listed. Very little variability was observed in the pollen sterility of the flowers of the same plant, as is shown by the small standard error. Clark and Fryer (1930) and Engelbert (1932) reported the same constancy of pollen sterility from plant to plant. Although the number of self-tripped flowers was not observed for this group, there is seen to be a definite correlation between the proportion of good pollen and of pod-setting. Plants 84–3 and 84–78, which set no pods, have a very low percentage of good pollen, while 84–11 and 84–88, plants which set pods well, have the highest percentages of good pollen.

It was noted also that the plants which had high percentages of sterile pollen (see Tables VI and VII) were deficient in amount of pollen as well, and that a high proportion of shrunken pollen grains seemed to be associated with faulty dehiscence of the anthers. The presence of these defects frequently results in failure of the stigma to capture any vital grains of pollen between itself and the standard when tripping occurs.

Table VIII. *Relation of pollen sterility to the number of seeds per pod.*

Plant type	No. of plants examined	Mean no. of seeds per pod	Mean percentage of good pollen
Parent plants	4 cuttings	3·3	87
L_1 plants	18	2·1	64
L_2 plants	15	1·7	45
F_1 hybrids	8	5·3	82

Table VIII relates to plants selected at random from the first and second selfed generations of a high pod-producer and shows that pollen sterility increased in each generation of inbreeding over that of the parent plant. The percentage of good pollen, however, was no greater in the hybrids than in the parent plants. The table also shows that the mean number of seeds per pod decreased in each succeeding selfed generation, but increased over that of the parents in the case of the F_1 hybrids, probably due to hybrid vigour.

Pollen sterility is thus seen to be a very definite factor in seed production, both in determining the pod-setting and in the number of seeds per pod.

DISCUSSION.

From our observations it would appear that seed production in alfalfa is dependent upon the occurrence of a train of events within the flower as follows: (1) abundant, viable pollen must be shed at the erect standard stage; (2) the staminal column must be released from the keel, *i.e.* the flowers must be tripped; (3) in the act of tripping, the column must strike the standard with sufficient force to rupture the stigma; (4) after tripping occurs, the proper moisture relationship for pollen germination and pollen tube growth must be maintained to effect fertilisation; and (5) after fertilisation the water metabolism of the plant must be such as to prevent ovule abortion.

All of the above prerequisites to seed production apply in the case of alfalfa plants generally, but with respect to the normally self-fertilised plants, described by Kirk and White (1933) and studied in the present investigation, there must also be automatic tripping of the flowers. This appears to take place when the dynamic force present in the staminal column is sufficiently greater than the static force present in the keel to cause the tripping mechanism to explode spontaneously.

Kirk (1933) has emphasised the fact that automatic tripping in his autogamous alfalfa plants is dependent on genetic factors and that these operate most effectively when they are closely associated in inheritance with a preponderance of favourable growth factors.

It has been shown that pollen sterility is associated with imperfect dehiscence of the anthers and that it lessens the chance of the stigma capturing viable pollen grains between itself and the standard, thus decreasing the number of seeds per pod. It is evident also that pollen is shed normally in the bud stages and that pollination occurs previous to tripping. The work of Clark and Fryer (1930), Engelbert (1932), and the studies reported here, indicate that individual plants produce consistently approximately the same amount of sterile pollen in different seasons.

Some doubt has existed in recent years as to the necessity of tripping, but the work reported in this paper indicates that tripping is essential for seed production. Only under field conditions which are very favourable for automatic tripping can ordinary alfalfa plants become self-fertilised. Our high seed-setting selections are much less exacting as to the environmental conditions which bring about self-tripping.

Martin (1915) showed that only under maximum atmospheric humidity can pollen germinate on a dry substratum. Such conditions rarely, if ever, exist in the field. Hence, to initiate pollen germination, moisture is required which is supplied by the rupturing of the stigmatic membrane. Furthermore, even if the humidity were such as to initiate pollen germination, our studies have shown that pollen tubes can only penetrate the stigma after it has been ruptured. Tripping is therefore believed to be a universal requirement for all alfalfa plant types.

The remaining requirements for effective fertilisation are dependent largely upon a favourable environment with respect to both soil moisture and atmospheric humidity. But even here plant selection may prove helpful. Torssell (cf. Engelbert, 1932) found that alfalfa strains differed in the degree of water resistance of the pollen.

SUMMARY.

This paper reports the results of a study of pollination and fertilisation in alfalfa. The experimental material consisted of plants of the Grimm variety and certain high seed-setting selections which are normally self-fertilised.

The high seed-setting types do not differ from the low seed-setting types with respect to the stage of anther dehiscence, amount and distribution of the pollen, or relative length of pistil and anthers. Anther dehiscence in both types regularly begins in the pointed bud stage and pollination is completed at the erect standard stage.

Pod-setting is shown to depend upon flower tripping. Tripped flowers set a high proportion of pods, while untripped flowers invariably wilt and drop.

The high seed-setting capacity of certain selected autogamous plants is largely due to the fact that fertilisation is accomplished by spontaneous tripping of their flowers.

Morphological differences between the tripping mechanism of high seed-setters and low seed-setters have been identified. Automatic tripping in the former is found to be more or less independent of environmental conditions.

Pollen germination occurred in 84 per cent. of the tripped flowers and in less than 1 per cent. of the untripped flowers examined.

Proof is offered that, in the act of tripping, the stigmatic surface is ruptured and that the released stigmatic content initiates pollen germination. Rupturing of the stigmatic surface is essential to penetration by the pollen tubes.

Pollen sterility is shown to be a factor in seed-setting in determining both the percentage of pods produced and the number of seeds per pod.

ACKNOWLEDGMENTS.

The writers wish to acknowledge their indebtedness to Dr L. E. Kirk, Dominion Agrostologist, and to Mr T. M. Stevenson, Agrostologist Specialist, Dominion Forage Crops Laboratory, Saskatoon, for kindly supervision throughout the course of these studies.

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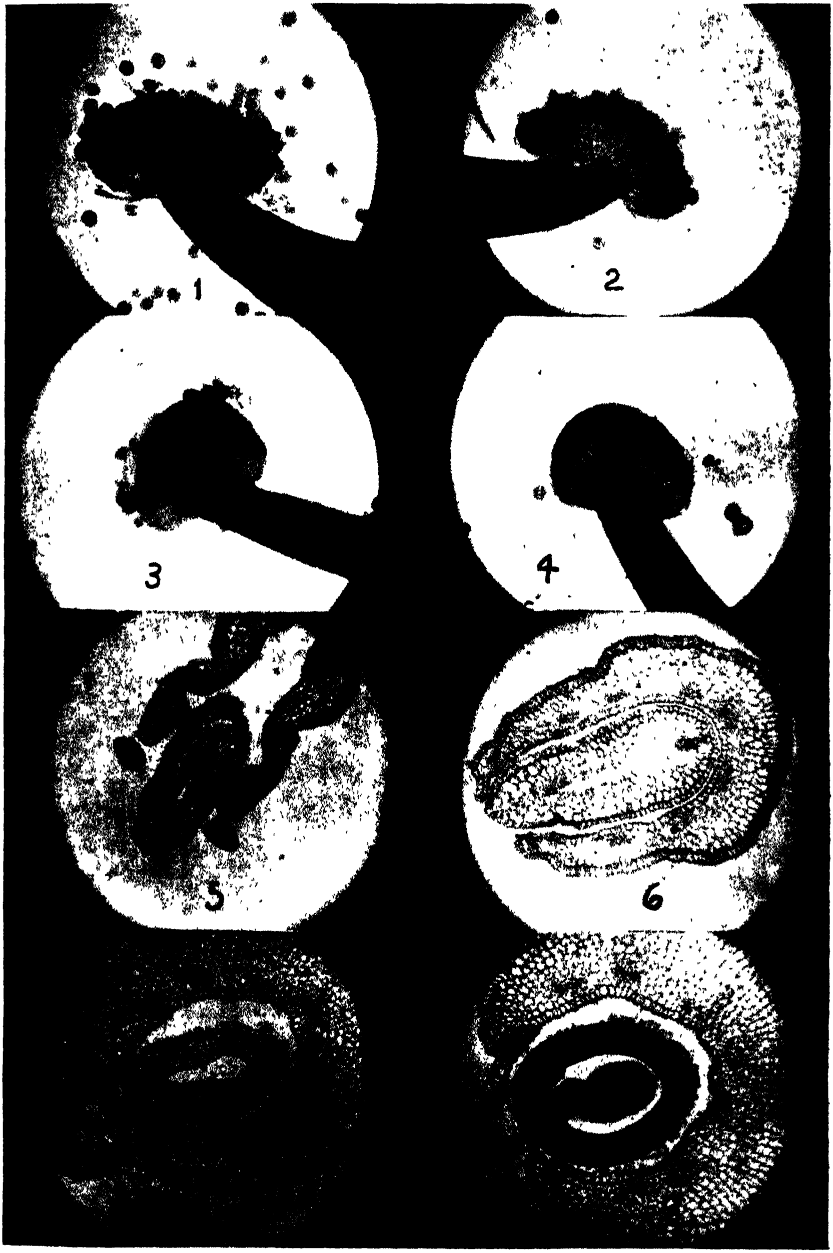
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EXPLANATION OF PLATE I.

Figs. 1–4. Side views of stigmas of alfalfa flowers.

Fig. 1. Stigma from tripped flower showing numerous pollen grains embedded in the stigmatic surface. Note the irregular flattened profile.

Fig. 2. Stigma from tripped flower in which the pollen distribution on the stigma is scanty.

Fig. 3. Stigma from untripped flower showing the pollen grains scattered loosely on the surface. Note the regular clear-cut profile.

Fig. 4. Stigma from tripped flower in which there has been a slight rupture of the stigmatic membrane at *a* and the contents have partly oozed out.

Figs. 5–8. Transverse sections through the staminal column and enclosed pistil at four different depths.

Fig. 5. Section just below the stigma at the point where the column is redividing into its constituent filaments. Note the cortex of thick-walled cells on the style.

Fig. 6. Section 2–3 mm. below 5 showing staminal column almost completely surrounding the style. The cortex of the stigma has been reduced to one thick-walled layer in front and two behind.

Fig. 7. Section just above ovary. In 7 and 8 the staminal column is seen to be wide in front with a slight groove in which the keel processes rest.

Fig. 8. Section through the ovary. The ovary is circular in cross-section.

(*Received June 25th,* 1934.)

A STUDY OF THE FASTING METABOLISM OF VARIOUS BREEDS OF PIG.

II. BODY TEMPERATURE MEASUREMENTS.

BY THOMAS DEIGHTON, M.A., D.SC., PH.D.

(*Animal Nutrition Institute, School of Agriculture, Cambridge University.*)

(With Three Text-figures.)

NORMAL TEMPERATURE.

THE normal body temperature of pigs is by no means so stable as that of many other warm-blooded animals. Tereg (1) gives the limits of normal variation as 101·3–104° F. (38·5–40° C.), while Stigler (2) quotes observations by J. Marek of 100·4–104° F. (38–40° C.), and of Cobbelt and Griffith 100·4–102·9° F. (38–39·4° C.). Richet (3) quotes 103·4° F. (39·7° C.) from Gley and Rondeau for the normal temperature level in these animals.

Our pigs had their rectal temperatures taken with an ordinary clinical thermometer (N.P.L. quality) inserted 8 cm. into the rectum. According to Stigler (4) the depth of insertion should be 10 cm., otherwise, it is alleged, the external temperature has some effect on the temperature recorded. To obtain this length of insertion a longer thermometer would have had to be used, and having begun the experiments with the ordinary type, some two years before the publication of Stigler's paper, it seemed best to continue the series of observations with the same technique. Whether any effect of surrounding temperature was observed or not is doubtful, as will be seen later, but what slight effect it appeared possible to trace, in view of the large normal variations with these animals, was of such a sign and magnitude as to be consonant with Stigler's findings.

Body-temperature observations were taken in all cases at the beginning of a calorimeter experiment, that is to say, after the animals had been living in a pen on the rations specified in Part I of this series (5), computed to supply maintenance and growth without appreciable fattening, for a period of at least a fortnight in the case of the younger pigs and extending to two or three months in some of the older animals. How far these temperatures may be regarded as *normal*, in view of the periodic 2–5 day fasts which the hogs underwent, is difficult to state with complete assurance. It appears to the writer unlikely that a comparatively short fast of this kind would have any large effect, extending over weeks or months, on the body temperature. As the results differ appreciably from those of previous workers, however, it is but right that this difference in treatment should be stressed.

The results obtained from 300 to 400 observations on the whole series of pigs experimented upon is shown by the top curve of Fig. 1. The pigs N–V showed no peculiarity in this regard and are therefore included. In all cases the pigs appeared to be in perfect health, no observation being included if this was questionable. The observations showed an appreciably greater range than those of former observers, viz. from 98·3 to 104·8° F. for adult pigs. It may be stated that quite a fair proportion of the very low values were observed with pigs A–M and W–Z (see Part I of this series) about whose normality in other respects there is no question.

As regards the possible effect of environmental temperature, this does not appear to have had anything to do with the abnormally low temperatures recorded from time to time, as will be seen from Table I.

Table I. *Total number of observations compared with the number showing normal temperatures below* 100° F. *at various environmental temperatures.*

Temperature of environment °C.	Total observations	Observations below 100° F.
18 or over	106	7
16–18	84	6
14–16	49	3
12–14	51	4
Under 12	50	2

From Table II it will be seen that this is also independent of the season of the year, and this more or less disposes of the possibility of a carry-over effect of previous exposure to a low temperature before being placed in the calorimeter (where the temperatures were taken) at the temperature indicated in Table I.

Table II. *Distribution of body-temperature observations below* 100° F. *according to season of the year.*

Season	Total observations	Observations below 100° F.
March, April, May	80	7
June, July, August	80	5
September, October, November	79	5
December, January, February	101	5

The drinking of cold water cannot have any effect in these cases, as the pigs were not given their morning meal until they came over to the calorimeter room and were then given their food to induce them to keep still while their temperature was taken; thus no time was allowed for any lowering by water drinking, as this takes some time to show itself in the rectum. In the pens in the animal house they had no access to water.

Hence we are driven to the conclusion that the lability of the normal body temperature of the adult hog is greater than has hitherto been supposed. Lanfranchi (6), in a highly interesting investigation, has found a considerable uncertainty in this regard in the temperatures of rats and guinea-pigs and is constrained to class them as "non-thermo-regulated animals" in contradistinction to rabbits, cats, dogs, and sheep which he calls "thermo-regulated". From the present results it appears that the pig must be classed with rats and guinea-pigs.

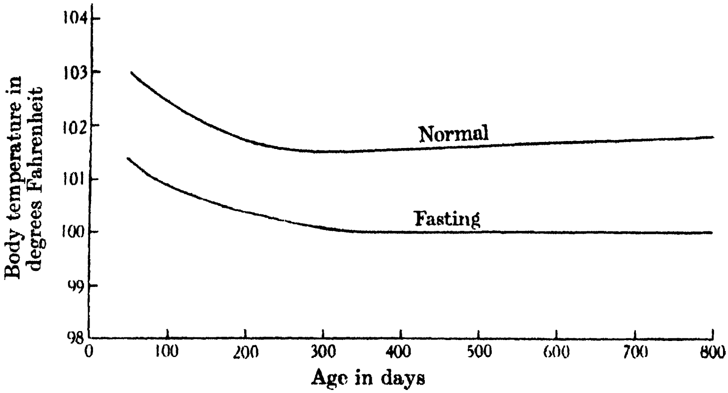

Fig. 1. Normal and fasting body temperature of hogs at different ages.

From Fig. 1 it seems that the temperature 102·6° F., which has hitherto been regarded as normal for the adult pig, is too high. A more suitable figure, at all events for hog pigs kept in sties, appears to be 101·7° F. Where the animals are free to move about a large enclosure it is conceivable that a higher figure may be justified, but unless the pig has just been exerting himself for some time it seems hardly likely that such a high figure as 102·6° F. would be attained, and of course the temperature so taken could not be classed as normal.

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[1] The general question of variation of body temperature in warm-blooded animals has been discussed by the writer in *Physiological Reviews* (1933), **13**, 427.

YOUNG PIGS.

With regard to the temperature of pigs when young, Stigler (2) refers to observations of Marek on piglings varying from 102·2 to 104·9° F. (39–40·5° C.), and others of Mihalyi from 104·7 to 105·8° F. (40·4–41° C.). The present observations indicate the limits for pigs 50–80 days old as 101·6–104·4° F. They fully confirm, however, that the body temperature in pigs as in other animals is higher in youth. The slight upwards trend of the line at the higher ages is probably of no import.

The higher body temperature in youth is doubtless the result of the higher metabolism in youth; but the connection is not obvious, except in a general way, when the individual observations are compared, probably owing to the variability of the normal temperature, but also probably from other causes, variability of vaso-dilatation, etc.

It seems possible that the higher metabolism of animals generally in youth may be merely a special case of the well-known fact that in animals of one species the temperature tends to be higher the smaller the animal even among adults. This may be a natural provision to keep vital parts at a proper temperature, taking account of the temperature gradients in the superficial layers.

TEMPERATURE IN FASTING.

The lower curve in Fig. 1 shows the observations of body temperature made on the hogs after they had been fasted for a period of two or three days in the youngest pigs, extended to 4 and 5 days as they grew older. There is the same variability, but the mean temperature, as indicated by the line, is lower by about 1·7° F. in the adult hog and by roughly the same amount in the young ones.

The only other observations I have been able to find of body-temperature measurements on fasting pigs are those of Johnson (7), who used only two pigs which soon ceased to be physiological. In one case he found a steady rise in body temperature as the fast progressed, in the others a fall followed by a rise.

The extent of the temperature fall observed is perhaps better illustrated in Fig. 2, in which the individual observations are classified according to the extent of fall (or rise) during fasting. It is at once clear from this that a definite fall does take place under these circumstances amounting to about 1° C. (say 1·7° F.). Moreover, the frequency distribution of the observations about the mode is of strikingly normal slightly asymmetric form, the deviations being of course far greater than could be ascribed to thermometer errors or errors of observation. Hence this normality of distribution has reference to the actual variations in the pig's temperature reduction in fasting above and below its modal value.

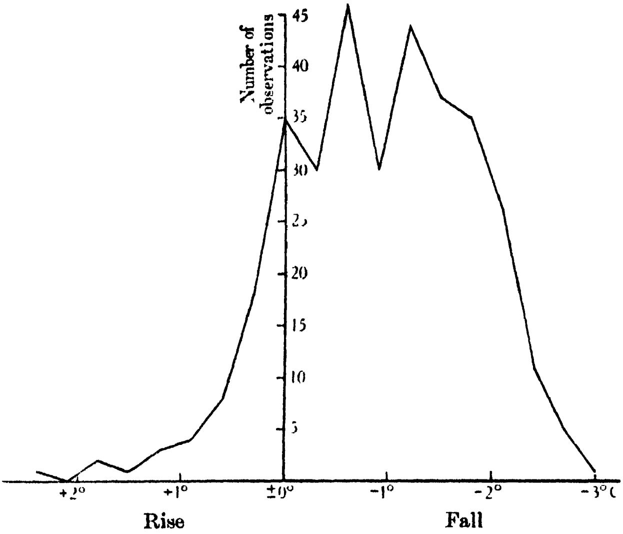

Fig. 2. Frequency distribution of body-temperature change in fasting.

DISCUSSION.

The present work makes a definite addition to the known facts regarding the course of body temperature in fasting. Investigations of other workers have led, and are leading, to résults which, as time goes on, point more and more to there being a real difference in the behaviour of different animals in this respect. Richet (*cit.* Lanfranchi), working with dogs, found a definite fall of about 1·5° C. in fasting; Ritzman and Benedict (8) found no such fall in sheep. Rabbits were investigated by Isenschmid (*cit.* Lanfranchi) who found a fall in temperature in fasting, and this has lately been confirmed by Tasaka (9), who also finds the body temperature of these animals more labile than that of man. Nichita and Mircea (10) find no body-temperature fall in fasting fowls, confirming Benedict, Landauer and Fox (11). On the other hand, Nichita and Iftimesco (12) find a distinct reduction in pigeons, while Chossat (*cit.* Richet) found no reduction in these birds, at best about 0·1° C. Richet (*loc. cit.*) mentions negative results of Martins with ducks, and of Bardier with geese. Similar negative results for man are recorded by Moüin and Maréchal (13) with Merlatti;

by Luciani (14) with Succi; and by Benedict (15) with Levanzin. All these were professional fasters. In human beings generally Benedict and Snell (16) found no reduction nor did Benedict, Horst and Mendel (17) in large rats. Although the majority of these results are negative, those of Richet, Isenschmid, Tasaka, and Nichita and Iftimesco are notable exceptions, and taken with the present results appear to show that there are some animals, of which the pig is clearly one, in which fasting induces a definite reduction of body temperature.

The *breed* of pig is apparently without effect in this matter.

The effect of individual idiosyncrasy within the boundary of a single breed is markedly shown in the case of pigs B and K. These were both Gloucester Old Spots, but while with pig B (which was exceptional) the average fall amounted to less than 0·1° C., with pig K it was more than 0·8° C.

From Fig. 1 it is clear that the fall in temperature is quite independent of age, and so, we may reasonably conclude, of length, weight and surface, each considered *per se*. There remain various other factors that might conceivably influence it—sex is not in question, since all the pigs were hogs. Breed has already been mentioned. Muscular effort, psychic state, fall in metabolism in fasting, weight for age, surface for weight, weight loss in fasting, environmental temperature, light and the drinking of cold water half an hour or so before the temperature measurement, have each to be considered.

The indications of the galvanometer curve give a good idea of the amount of muscular energy expended by the pig during the hour before the temperature was taken, and an examination of these records in those cases in which the fall of temperature was more than 2° C., and the temperature at entry not unduly raised shows that out of twenty available observations the pig was quiet in twelve cases, moderately disturbed in six and violently disturbed in one. One case was doubtful, as, although the pig appeared to have been quiet, the heat registered was much higher than during the hour previous to that concerned. A corresponding examination of the curves for those cases in which a rise, or at least no fall, in temperature was observed, and in which the temperature at entry was not unduly lowered, yields thirty-six observations comprising four in which the pig was quiet, twelve with moderate movement and nineteen in which the animal was violently disturbed. It is therefore clear that there is a definite connection between the muscular exercise taken within the hour previous to the temperature measurement and the fall in temperature of the hog observed at the end of the experiment, but naturally this does not imply anything more than that exercise by raising the body temperature tends to minimise a fall present for other reasons.

It is clear that there is no reason to change the figure 1·7° F. for the average temperature fall in fasting on account of the inclusion of a certain number of observations in which the fall is reduced or cancelled by bodily disturbance of the pig, since the figures for temperature at the beginning of the experiments doubtless also contain about an equal number of cases where a rise has taken place by bodily exertion.

The psychic state of the pig appears to have some effect on the body temperature, excitement seems to raise this, but the effect is not easily separated from that of muscular energy, as when mentally excited or unaccustomed to the experimental procedure animals tend to be very restive. In such cases it has been our custom to measure the temperature again when giving the last meal before the fast begins. The pig has then been inside the instruments for some hours. Such readings are always normal, and it is these which appear in the observations recorded in Fig. 1 in such cases, not the artificially raised morning temperature.

A scatter diagram of the fall in body temperature during fasts against the percentage excess of metabolism after a 12-hour fast over the determined fasting katabolism shows no evidence of the existence of any connection between these two factors such as seemed probable as the result of experiments on two pigs previously published by the present writer (18). In so far as individual observations are concerned, therefore, we must conclude that the suggestion there made that the metabolism was slowed down by the reduced body temperature, in cases where this is low, in accordance with van't Hoff's Law, does not represent the facts of the case. A similar state of affairs obtains where the results for each pig are separately averaged. Finally, if the observations are grouped according to the extent of temperature fall, rejecting those cases in which a rise of temperature was found as in Table III, there is still no significant relation, as the standard error of the means in all cases exceeds 2·00 even if one or two extreme values be rejected by Chauvenet's or Pierce's

Table III. *Relation between body-temperature fall and extent of fall of metabolism from 12 hours' fasting level to fasting katabolism.*

Range of body-temperature fall °C.	No. of observations	Mean metabolism fall % of fasting katabolism
0·0–0·4	45	36·94
0·5–0·9	58	40·66
1·0–1·4	54	43·36
1·5–1·9	58	41·50
2·0 and over	37	42·41

criterion. Hence we appear to be justified in asserting generally that no relation between these two factors exists in the sense formerly suggested.

Coming now to the question of the possible influence of weight for age, the data for those pigs of the groups A–M and W–Z which were over the mean weight (A–M) and those which were under this have been separately averaged with the following result:

	Pigs *over* mean wt. for age	Pigs *under* mean wt. for age
Mean fall in body temperature	0·94° C. (77 observations)	0·87° C. (122 observations)

The standard error of the means being about equal to the difference between the two figures, weight for age would appear to be without effect on the temperature fall with which we are dealing.

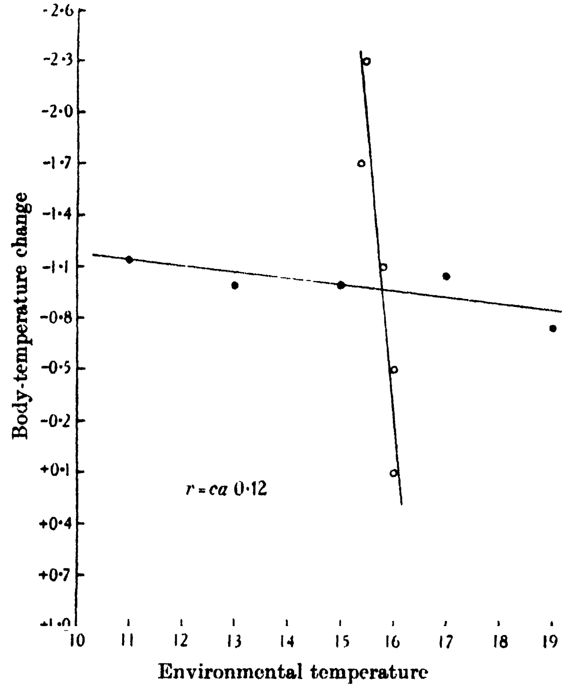

Fig. 3. Regression lines of environmental temperature on body-temperature change and *vice versa*.

It seemed possible that the fall might be related to the ratio borne by the surface to the two-thirds power of the weight. The data in this case are rather more restricted, pig L being the first of the series on which any measurements of body surface by the photographic method (19) are available, the surface of previous pigs having been computed by a formula involving the weight. From the figures available there does not appear to be any connection whatever between these two factors, this ratio being apparently much more closely related to the individuality of the pig. The matter may be returned to in a later paper should circumstances warrant it when the surface area computations are completed.

A scatter diagram of the fall in body temperature plotted against weight loss in fasting, whether net (see introductory paper of this series) or gross, shows no relation between these two.

There remains the possible effect of external factors, namely environmental temperature, light and the drinking of cold water.

The effect of the first of these has been investigated by means of a correlation table over the intervals 10–20° C. and body temperature change from +0·4 to −2·6° C., in which ranges a sufficiently large number of observations was available to enable one to use 2° intervals in environmental temperature and 0·6° intervals for body temperature. The regression lines are shown in Fig. 3, and it is clear that the correlation is very slight ($r = ca.\ 0{\cdot}12$). This small connection may quite well be due to the 8 cm. insertion instead of 10 cm. as required by Stigler's findings, combined with any small effect which may be present in cases where water at the temperature of the surrounding air was drunk within an hour or so before the temperature of the pig was measured. It would in any case be rash to conclude on the present results that body temperature is at all affected by changes in environmental temperature.

Table IV. *Body temperatures of pigs fasted in the animal house in daylight.*

Pig	Temperature at beginning of fast °F.	Temperature after 40 hours' fasting °F.
U	101·6	100·2
V	102·1	100·9
W	100·5	98·6
X	100·1	96·8
Z	102·2	101·7

In view of the large number of results published on other animals in which practically no temperature reduction occurred in fasting, it seemed possible that the results we have obtained might be traceable to the fact that the fasts were always conducted in darkness. Accordingly the pigs which remained were fasted all together in their pens, so that psychological excitement due to hearing other pigs being fed might be avoided. The results are shown in Table VI.

It is clear that conducting the fast in daylight does not alter the picture in any way. The reductions in temperature varying with the pig from 0·5 to 3·3° F. are quite comparable with the reductions obtained in the calorimeters.

Finally, as regards the drinking of cold water half an hour to an hour before the temperature measurements. This was uncontrolled, the animals having been allowed unrestricted access to water, and may in consequence have affected the results slightly. The water would in all cases have been at the environmental temperature, and if the effect is present in some of the pigs it would also operate, as suggested above, to increase any negative correlation between fall in body temperature and environmental temperature which may be present from other causes. It is, however, quite unlikely to have come into play in more than a few cases, and in view of the results of Isenschmid (*cit.* Lanfranchi) and those of Hashimoto (20), both on men, is unlikely to have increased the body-temperature fall more than fractionally in those cases where the effect was present. What the actual cause of the fall in temperature may be remains undiscovered, but as the result of this investigation we know a number of things which are not the cause of it.

The writer is disposed to think that the hog falls into the class of what Lanfranchi calls "non-thermo-regulated animals"—incompletely thermo-regulated would perhaps be a better term. It would be interesting to know whether guinea-pigs exhibit a temperature fall in fasting, since they are also incompletely thermo-regulated; rats apparently do not, according to the paper of Benedict, Horst and Mendel already cited.

SUMMARY.

The normal body temperature of the pigs used in these experiments showed an appreciably greater variability than has hitherto been considered normal for pigs, the limits being 98·3–104·8° F. and the variation is independent of environmental temperature, or season of the year. The average normal temperature of the adult hog is 101·7° F.

In fasting for from two to five days a reduction of body temperature was noted in a large majority of cases, amounting on the average to 1·7° F.

Whether the normal or fasting figures are taken, the mean body temperature is higher in youth (50 days) than in the adult hog by about 1·5° F.

It is concluded that the reduction of body temperature in fasting in hogs is independent of breed, age, weight, surface, weight for age, surface

for weight, length, weight loss in fasting, fall in metabolism in fasting, environmental temperature, and light.

Individual differences are marked and mental excitement appears to have a noteworthy temporary effect, as also has the amount of muscular energy expended within an hour or so before the measurements were taken. Drinking of cold water before the temperature measurement may also affect the fall slightly by analogy with human beings, but the effect is unlikely to be great. The actual cause of the fall in temperature does not appear.

It is concluded that hogs, like rats and guinea-pigs, are incompletely thermo-regulated animals.

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(*Received July 1st*, 1934.)

STUDIES IN TROPICAL SOILS. INCREASE OF ACIDITY WITH DEPTH.

By H. C. DOYNE, M.A., A.I.C.

(Department of Agriculture, Nigeria.)

In recent years the subject of tropical soils has received much attention and it is now realised that preconceived ideas of soil formation and soil fertility, which were for the most part derived from the study of European soils, have to be reconsidered when applied to the Tropics.

It is somewhat surprising that little appears to have been written about a peculiar and common, although certainly not invariable, feature of many tropical profiles, viz. the increase of acidity with depth.

This fact has been mentioned by Gracie and Le Poer Trench (1) in describing possible causes of infertility in Kenya, and by Doyne and Watson (2) in the study of the Ilepa profile in Nigeria. Robinson (3) gives examples and analyses of soils from East Africa obtained from Milne, all of which show this feature.

In this paper some account is given of the chief variations in the change of acidity with depth found in the study of many profiles in Nigeria. The explanations offered are tentative, and there are instances of profiles, not recorded here, which cannot be satisfactorily explained.

The first type is that in which the acidity increase is confined to the horizon immediately below the surface, and subsequent horizons show no regular increase or decrease. This is the commonest feature in Nigerian soils, and the reasons suggested may also apply to other types to be considered later. The essential condition seems to be that the surface vegetation has been undisturbed for some considerable time, although the acidity and increase of acidity with depth is by no means an invariable accompaniment of these conditions.

It has been stated by Mohr (4) that at a temperature of about 30° C. the microbiological destruction of organic matter is sufficient to keep pace with additions of organic matter resulting from plant growth assuming that other conditions (air and moisture, etc.) are suitable. The plant roots take up mineral matter which is ultimately deposited on the surface by the leaf fall. Owing to the rapid decay of this plant refuse, the bases combined with the humic colloidal matter are set free, and the base status of the surface soil is increased at the expense of the horizon from which the plant roots draw their food. As would be expected, the difference in base status between surface and subsoil is often more pronounced under high forest or "old" bush than under shallow-rooted grass vegetation; and on permanently cultivated soils it is doubtful if the difference occurs at all. Examples are given in Table I of two profiles of a similar soil derived from a sedimentary formation, one profile being taken from high rain forest and the other from a grass area close to it, which probably had been cleared of forest and cultivated in by-gone ages and long since reverted to grass. The rainfall of the locality is 80–90 in. and the latitude 6° N.

Table I. *Profiles from high forest and grass, Benin Province, Southern Nigeria.*

	Mechanical analysis (no stones)				Exchangeable bases mg. equiv. %		
Depth in.	Coarse sand	Fine sand	Silt and clay	*p*H (*a*)	Absorbed (*b*)	Present (*c*)	Saturation % (*d*)
High forest soil (carbon in first foot 0·667 %).							
0–12	85·7	5·1	9·2	5·1	2·95	1·60	35
12–24	76·2	8·3	15·5	3·6	4·0	0·08	2
24–36	76·7	6·8	16·5	3·6	3·3	0·16	5
36–48	74·3	6·2	19·5	3·7	3·0	0·08	3
48–60	73·1	6·6	20·3	3·7	2·9	0·16	5
Grass soil (carbon in first foot 0·495 %).							
0–12	85·7	6·2	8·1	4·5	2·4	0·48	17
12–24	73·0	10·9	16·1	4·2	3·0	0·24	7
24–36	68·1	11·0	20·9	4·1	3·0	0·40	12
36–48	69·0	9·2	21·8	4·1	3·0	0·08	3
48–60	67·5	8·6	23·9	4·2	2·4	0·08	3

(*a*) Method of Kuhn (5).

(*b*) Para-nitrophenol method of Schofield (6).

(*c*) Method of Rice Williams (7).

(*d*) $\frac{c \times 100}{b + c}$.

The mechanical analysis is included in order to show the similarity of the two soils, which had a uniform deep red colour. In both profiles there is a definite drop in *p*H value at a depth of 1 ft., but this drop is greater for the soil under high forest. Further, while the forest soil has only a little more carbon in its first foot than the grass soil, it contains more than three times the amount of exchangeable bases and its *p*H and saturation are considerably higher. Apparently the more extensive root range and the greater amount of leaf fall of the forest caused a greater transfer of bases from subsoil to surface soil. It is certainly a well-known fact that cultivation of a forest or high bush area is more successful than cultivation of an area under grass.

A similar increase of acidity immediately below the surface, but due to very different causes, takes place on tidal mud flats some time after mangrove forests are cleared. Such profiles were studied in some detail in Sierra Leone by Doyne and Glanville (8). The primary cause of this appears to be the exchange action of the sodium chloride in sea water liberating hydrogen ions in the decaying mangrove roots below the surface and the formation of a sodium clay on the freshly deposited surface mud. Such soils are obviously of a very specialised type and consequently will not be considered further.

In the next type to be considered there is an increase in acidity to a definite depth below which there is a regular decrease. This seems to occur on fairly mature profiles under conditions of a short period of intense rainfall followed by a long period of extreme drought. It was first noticed on the sandy transported soils which cover a large area in the north of Kano province in Northern Nigeria (latitude 12·5° N.). The rainfall of this district is about 25 in. and is confined to the months of June, July and August. The vegetation is largely thorn bush. The profile samples were taken at the end of the dry season in May, in places where well boring was in process.

Table II. *Profile from Hadeija Emirate, Kano Province. Locality—Antukwa.*

Depth in.	Description	*p*H	Exchangeable bases mg. equiv. % Absorbed	Exchangeable bases mg. equiv. % Present	Saturation %
0–12	Grey sand	6·1	0·50	2·12	81
12–24	Brown grey sand	5·3	0·70	2·00	74
24–36	Light brown sand		0·80	1·68	68
36–42	Yellow sand	1	0·65	1·28	66
42–53	White sand with brown streaks	3	0·35	0·80	70
53–63	Soft, floury, white sand	6·8	0·05	0·32	87
Below 63	Hard whitish clay	6·9	0·05	1·27	96
At 73 ft.	Red clay	7·8	Nil	4·14	100

No carbonate was present and the soluble salt content was negligible.

A further example at Meko in South-Western Nigeria is given in Table III. The profile consisted of an almost black sandy surface soil

Table III. *Profile from Meko, Abeokuta Province. Latitude* 7·5° N.

Depth in.	*p*H	Exchangeable bases mg. equiv. % Absorbed	Exchangeable bases mg. equiv. % Present	Saturation %
0–4	5·9	1·5	3·18	68
4–8	5·7	1·7	2·06	55
8–16	5·0	2·1	1·79	46
16–32	5·5	2·6	2·78	52
32–48	5·9	2·1	3·23	61

becoming redder and heavier as the depth increased. There was no sign of decaying rock or mottled clay in any of the horizons. The rainfall of the district is about 40 in., and the area consists of a high plateau with a low humidity in the dry season.

A possible explanation for the two profiles in Tables II and III may be as follows. Both areas have a very pronounced dry season when evaporation is high. During the brief and very heavy rains, the soil becomes thoroughly saturated. In the dry intervals, the displaced bases from a definite depth (depending possibly on the permeability of the soil and the temporary water table), will be drawn up to the surface; below this depth the leaching action of the water predominates. Thus the horizon at which the pH and saturation are lowest is being denuded of its bases in both directions, downward by leaching and upward by evaporation. It is noticeable that this "desaturated" horizon is considerably lower in the very sandy northern soil (Table II) than in the heavier southern soil (Table III).

Finally, the type must be considered where the acidity continues to increase without a break into the decomposing rock. This is typified by the "Ilepa" profile described in a paper (2) referred to above. Such profiles occur essentially with immature soils derived from an igneous, acidic rock, the conditions apparently being alternate wet and dry seasons with a uniformly high temperature of about 80° F.

The profile consists of a brown sandy soil passing into, and sometimes mixed with, a gravelly horizon of concretions and quartz fragments. This gradually merges into a red clay which becomes mottled with white streaks which tend to increase in amount with depth. The mottled red and white clay (the "Ilepa" proper) frequently contains veins of quartz running through it and shows definite evidence of a rock decomposing *in situ*.

Evidence showed that the concretions which occur at the upper limit of the "Ilepa" zone and consist of ferric oxide with some alumina and oxides of manganese were derived from the mottled clay or "Ilepa" zone and were due to a rise of "mobile" iron, etc., formed under the acid conditions which prevail at that depth and deposited in a horizon with a higher base status.

A typical "Ilepa" profile is shown in Table IV. This was taken from a railway cutting at the end of the rains in October when conditions which should favour downward leaching of bases would presumably be at their maximum.

The highly acid conditions in the deep horizons may be due to the

Table IV. *"Ilepa" profile. Ibadan loco. sheds. Rainfall* 55 *in. Latitude* 7·5° *N.*

Depth in.	Description	*p*H	Exchangeable bases mg. equiv. %		Saturation %
			Absorbed	Present	
0–8	Surface soil	5·5	1·75	1·88	52
8–16	Concretion-horizons	5·3	2·35	2·4	50
16–24		5·2	2·90	2·88	50
24–34		5·2	3·30	2·80	46
34–46	Mottled clay ("Ilepa") and decomposing rock	4·7	4·20	2·32	36
46–58		4·5	4·95	1·76	26
58–70		4·4	5·70	1·48	21
Below 70		4·2	8·0	1·92	19

percolating water being held up in the temporarily waterlogged and rapidly decomposing rock. As the rock is acid the resulting clay will have a low base status. In the frequent dry intervals when evaporation is rapid the displaced bases will rise into the higher zones together with the mobile sesquioxides formed by the acid conditions. The latter are deposited and irreversibly desiccated in the concretion horizon which has always a higher base status than the more clayey ("Ilepa") layer below.

Thus the process must be due to a heavy but intermittent rainfall, rapid percolation through the upper horizons and temporarily waterlogged conditions in the decomposing rock area. Evaporation exceeds percolation in this area so that there is a continual upward movement until the rock reaches the limit of its decomposition.

Unfortunately the decomposing rock has never been explored to a greater depth than about 10 ft. from the surface. It is obvious, however, that a point must be reached somewhere when the acidity becomes stationary or decreases, but this has not yet been found.

A mature or degraded "Ilepa" profile consists of a deep accumulation of concretions which form a solid conglomerate in the upper horizons and pass directly into a crumbling rock brash in the depths. The true "Ilepa" horizon is absent and the concretion horizon contains a fair amount of clay but the rock brash does not. Such a profile shows no increase of acidity with depth. It may be regarded as "telescoped Ilepa" profile produced where there is only a small amount of clay-forming material (felspars etc.) in the rock itself and when the intermediate "Ilepa" clay had all been converted into concretions. More rapid desiccation may also be involved and it is significant that such profiles are more common in Northern Nigeria, where the humidity is considerably less, than in the south.

SUMMARY.

1. Increase of acidity with depth is a common feature of Nigerian soils.

2. Examples and possible explanations are given for three types of acidity increase with depth. In the first, the greater acidity is confined to the depths immediately below the surface and is possibly dependent on a permanently undisturbed vegetation. In the second, the acidity continues to increase to a certain depth below which there is a regular decrease. This is more evident on mature soils, and may be dependent on a short but intense rainfall followed by a prolonged drought. In immature soils, derived from igneous acidic rocks under alternate wet and dry seasons, the acidity increases steadily with depth into the decaying rock mass.

ACKNOWLEDGMENTS.

The author wishes to express his thanks to Mr O. T. Faulkner, C.M.G., Director of Agriculture, Nigeria, for much valuable advice and criticism, to the Forestry and Geological Survey Departments in Nigeria for help in obtaining the profiles from Benin and Kano provinces, and to the African Staff of the Moor Plantation Chemical Laboratory, Messrs J. K. Ladipo, T. O. Bademosi, C. O. Phillips, and E. Morakinyo, who helped to carry out some of the analyses.

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(*Received November* 10*th*, 1934.)

SOME EFFECTS OF RADIOACTIVE MUD UPON GERMINATION OF SEEDS AND GROWTH OF SEEDLINGS.

By LÁSZLÓ HAVAS.[1]

(With Plates II and III and Six Text-figures.)

CONTENTS.

		PAGE
I.	Materials and method	198
II.	Experiments and results	199
	(*a*) The effects of remote radiation upon resting seeds, prior to sowing	199
	(*b*) (i) Direct and indirect effects upon the germination and growth of seeds	203
	(ii) The screening effect of interposed lead sheet	207
	(*c*) The effect of radiation upon seeds bathed in dyestuff solutions	208
	(*d*) Soil experiments with radioactive mud in pots	210
III.	Discussion	212
IV.	Summary	213
V.	Acknowledgments	214
VI.	Appendix: Analytical data concerning the soil and radioactive mud	214
	References	215

I. MATERIALS AND METHOD.

THE experiments described in this paper were carried out as an extended repetition of those of Chavan and Havas (1919). At that time, in spite of the outstanding pioneer work of Molisch (1912) and of Stoklasa (1912, 1913), no clear results had emerged from such work on the effects of radioactive salts, and, as far as the author is aware, no attempt had been made to examine the phytobiological influence of radioactive mud.

In the present work, which involved the examination of some 14,000 seeds, the seeds were chosen so as to be as far as possible homogeneous as regards age, source, size, ripeness, and water content. Fourteen Hungarian varieties, of eight common agricultural species, were used, of harvests 1932 and 1933. For determination of the effects of the radioactive mud (hereafter called mud for brevity) the following criteria were used: rapidity of germination, growth of sprouts, and growth of roots. This last was somewhat approximate, since the seeds were sprouted on the surface of filter paper, radioactive mud, or ordinary earth, in which they had a tendency to bury themselves if it was possible. On account of the absorption of radiation by water no water cultures were made. Apparatus and the water used for moistening the seeds were sterilised.

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[1] The work described in this paper was performed at the Hungarian Biological Research Institute, Tihany, Hungary.

All the seeds and also the mud were of Hungarian origin, and the experiments were performed at the Biological Institute, Tihany, Hungary. The mud was supplied by courtesy of the Héviz Baths; it consisted principally of the sediment of thermal springs, mixed with humic material (see Appendix).

II. EXPERIMENTS AND RESULTS.

(*a*) *The effects of remote radiation.*

This experiment aimed at detecting the possible stimulatory or adverse effects upon germination after seeds had been exposed in the resting condition to a preliminary irradiation from mud.

Technique. The dry, untreated seeds were enclosed in a sealed glass cylinder, which was buried near the centre of a sack containing about 25 kg. of mud, and allowed to stay there during periods of from 24 hours to 7 days. A control, consisting of similar seeds enclosed in a vessel of the same capacity (80 cm.^{3}) was kept in the dark but not irradiated.

Seeds were germinated in flat earthenware dishes, diameter about 25 cm., which had been filled with a mixture of garden earth (see Appendix for analysis) and an equal weight of washed quartz sand. After 3 or 4 days in an incubator at about 20° C., the seedlings were transferred to a greenhouse. Water was supplied as needed.

All the seventeen kinds of seeds mentioned in the Appendix were used in this experiment, but the results obtained with radish seeds were not conclusive on account of their naturally rapid germination. All the other seeds were affected by, and showed marked response to, irradiation, but for reasons of space only a few typical results can be selected for tabular and diagrammatic representation.

Table I is a summary table showing the stimulating (indicated by a +) and repressive (−) effects upon seeds previously subjected to irradiation in a closed cylinder, as compared with the controls. The results of Rivera (1930) indicate that oxygen was not a limiting factor in such circumstances. Text-figs. 1 and 2 show comparative germination after 1 and 7 days' irradiation. Text-figs. 3 and 4 show the lengths of the shoots in millimetres in representative experiments after 1 and 7 days' irradiation, respectively.

Table I. *Summary table showing stimulated (+) and repressed (−) growth of seedlings sprouted after exposure to remote radiation for the times stated.*

	Time exposed to remote radiation before sowing												
	1 day					2 days			3 days		7 days		
No. of exp. ...	III	IV	V	IX	X	VI	VII	XII	II	II*a**	I	I*a*	XI
Triticum	+ 6	+ 9	+11	+39	+ 5	− 8	−10	− 5	−12	−54	+ 7	−11	−24
Avena	+ 8	+ 7	+12	+31	+ 9	− 9	− 9	+ 5	+ 7	−41	+ 8	−13	−21
Pisum	−14	+27	+46	+20	+10	−40	−33	− 6	−17	−30	− 7	− 3	−19
Phaseolus	+14	+10	+11	+23	+14	−39	−53	−12	−42	−45	—	−33	−18
Soja	0	+17	+ 9	+19	+ 5	−17	−26	0	—	−71	−46	−31	−15

Results are expressed as percentage increase or decrease of mean shoot length, the average shoot length of a simultaneous control being taken as 100.

* Seeds steeped before irradiation.

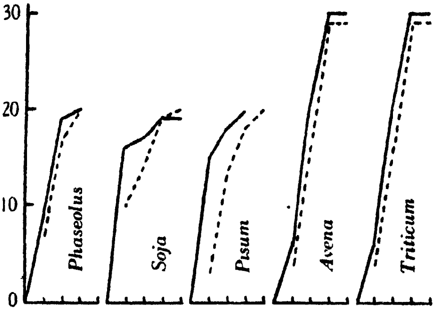

Fig. 1. Effects of remote radiation upon germination: 24 hours' irradiation before sprouting. ---- not irradiated; —— irradiated.

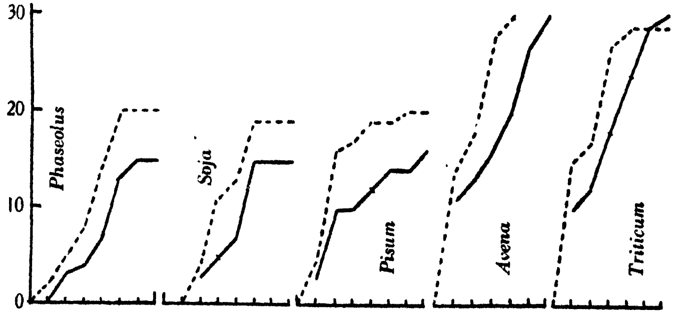

Fig. 2. Effects of remote radiation upon germination: 7 days' irradiation before sprouting. ---- not irradiated; —— irradiated.

In Text-figs. 1 and 2 only the uppermost curve is taken to the origin. For wheat and oats thirty, and for the other species twenty seeds were tested.

It will be seen that 24 hours' irradiation led to a notable stimulation of germination and especially of growth. After 48 hours' irradiation, however, a retardation of growth occurred, except in one experiment with oats. The same remark holds for the only series of dry seeds examined after 3 days' irradiation, which depressed the growth of the other seedlings. After 7 days' irradiation the growth of all seeds, except one sample of oats and one of wheat, was depressed.

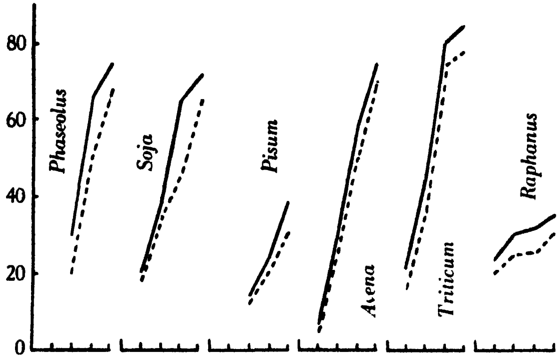

Fig. 3. Effects of remote radiation: shoot length of seedlings irradiated as seeds for 24 hours before sprouting. Average values in mm. from twenty or thirty seedlings. - - - - not irradiated; —— irradiated.

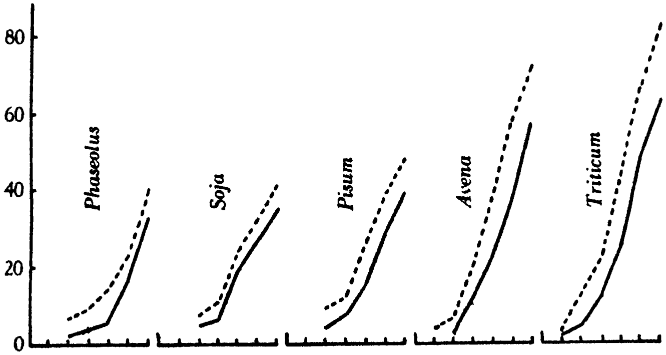

Fig. 4. Effects of remote radiation: shoot length of seedlings irradiated as seeds for 7 days before sprouting. Average values in mm. from twenty or thirty seedlings. - - - - not irradiated; —— irradiated.

The extent of the depressive effect was much affected by temperature, and at any one temperature was not clearly proportional to the length of time during which the seeds were exposed to radiation, but such irregularity is only to be expected of biological material. Two facts emerge, however: irradiation under these experimental conditions led to stimulation of growth, provided the irradiation did not exceed some period of between 24 and 48 hours; after this limiting time had been exceeded, further irradiation had an approximately constant effect in repressing germination and growth. This repressive effect is not lasting but related to a latent period after the expiration of which new growth begins (Rivera, 1928).

Effect of variation in experimental conditions. When the amount of mud was reduced from 25 to 4 kg., 4 days' irradiation produced a slight increase in the shoot lengths and had no effect upon germination of any of the seeds.

The results for *Pisum* given in Table I refer to pea 15, but in experiments with pea 14 a stimulatory effect of radiation was noted. Other apparent irregularities were noted in regard to oats and wheat, the fresher seeds (1933 harvest) being more adversely affected by radiation than seeds a year older. This finding suggested that the water content of the seeds might have a bearing upon the question of the effects of radiation. Some seeds were steeped for 24 hours in 0·1 per cent. aqueous sodium chloride solution before being put into the cylinders for 3 days' irradiation; the results are summarised under Exp. II*a* in Table I. It will be seen that the injurious effect of irradiation was greatly increased by the steeping, and this was true both for germination and growth (Stein, 1922; Reich, 1926; Ancel, 1927; Johnson, 1928). Lallemand (1930, 1932) and Lambert (1932), however, did not believe that the higher water content of freshly harvested seeds promoted a readier sensitiveness to radiation.

Certain inconsistencies in the comparative behaviour of the seeds may reasonably be referred to varietal differences and to variation in size, ripeness and thickness of coat of individual seeds (Montet, 1932). Detailed discussion therefore seems out of place.

The anatomy of seedlings from seeds previously irradiated for 24 *hours.* The macroscopic appearance of those plants which had been subjected as seeds to 24 hours' irradiation showed, in comparison with the controls, greater thickness of the sprouts and a constantly greater degree of turgescence. Phenomena such as have been called radiomorphoses (Caspari, 1926; Stein, 1927; Johnson, 1931) were not frequent in the present experiments; only slight changes in the distribution of the coloured constituents were noticed. Pale streaks and circular spots appeared on the leaves in Exps. I, I*a*, II*a* and XI only.

(b) (i) *Direct and indirect effects upon the germination of seeds.*

These effects were the subject of experiments under varied conditions. In the majority of experiments Petri dishes were used, containing about 70 gm. of radioactive mud in a layer 17–18 mm. deep. Seeds to the number of from seventeen to forty, were placed either directly upon the surface of the mud or upon moistened filter paper supported by a clock glass or Petri dish which was laid over that containing the mud. The total exclusion of emanation was not aimed at. Controls were prepared with sand or earth or with nothing in place of the mud. In most experiments the sand and water were sterilised, to avoid the disturbing influences of algal and mould growth.

Additional experiments were performed with quantities of about 170 gm. of mud contained in glass vessels 12 × 6·5 cm. and 5 cm. deep. (In a few cases flower-pots filled with mud were used (see Plate III *a*).) For the examination of the effects of small quantities of mud in intimate contact with seeds, about 1·2 gm. was powdered and strewn over the surface of moistened filter paper upon which the seeds had been sown. In these experiments (nos. 9 and 10) sterile washed sand was sprinkled as a control.

The seeds were kept in an incubator at 22–28° C., moisture being conserved by a glass cover over each vessel. The cover was kept on until the sprouts touched the cover (usually this happened within 2 days); the cover was then removed, but the seeds were not removed from the incubator until the fourth day, when they were put in the greenhouse and occasionally sprinkled. No nutrient substance was supplied and, as in Section (*a*), the experiment came to a term when the reserves of the seeds were exhausted.

A glance at Tables II, III and IIIA and Plates II, III *a* and Text-fig. 5, is sufficient to show that under the usual conditions of this set of experiments there was a quite general stimulation of germination and of the growth of shoots and roots. Plate III *b* shows the effect of 3 days' irradiation of soya beans laid directly upon mud.

The degree of stimulation depended upon whether the seeds were placed actually in contact with the soil or supported above it. Towards the end of the experimental period there was, in many cases, a tendency for the controls and treated seeds exposed on filter paper to yield the same measurement; this was of course an expression of the fact that, in the absence of added nutrients, growth, if not repressed by lethal effects of radiation, was conditioned by the reserves of the seed.

Table II. *Summary table showing mean percentages of germination on moist filter paper (control), and in and above radioactive mud, for the first 5 days of observation, over all experiments on the five species named.*

		Day				
	Germinated ...	1	2	3	4	5
Triticum	(*a*)	17	68	78	88	96
	(*b*)	39	88	100	—	—
	(*c*)	50	78	90	100	—
Avena	(*a*)	24	79	92	—	96
	(*b*)	79	99	100	—	—
	(*c*)	81	96	96	—	96
Pisum	(*a*)	6	25	49	71	91
	(*b*)	19	47	96	98	100
	(*c*)	18	56	90	98	100
Phaseolus	(*a*)	12	41	65	74	97
	(*b*)	34	47	65	82	71*
	(*c*)	12	38	41	59	74
Soja	(*d*)	0	10	28	34	64
	(*c*)	14	34	68	98	100

(*a*) On filter paper.
(*b*) Over radioactive mud.
(*c*) In radioactive mud.
(*d*) In garden soil (control).

* Decrease due to removal of mouldy seeds. Some of the other figures are affected by decrements due to the same cause.

Table III. *Germination.*

	Triticum								*Avena*						
N ...	25		40			40			30			30		
n ...	(1)		(3)			(4)			(7)			(8)		
D ...	Apr. 25		Apr. 29			Sept. 26			June 5			June 13		
Day	S.C.	M.	C.P.	O.M.	M.	C.P.	O.M.	M.	C.P.	O.M.	M.	C.P.	O.M.	M.
1	12	14	10	25	26	10	17	14	12	28	26	7	23	24
2	17	24	33	39	38	19	31	24	22	30	30	25	29	29
3	—	—	36	40	39	31	40	33	27	—	—	29	30	—
4	—	—	—	—	—	37	—	40	—	—	—	—	—	—
5	18	24	37	—	40	40	—	—	30	—	—	—	—	—
d	*12*	*6*	*11*	—	—	—	—	—	—	—	—	—	—	—

	Pisum						*Phaseolus*			*Soja*		*Beta**				
N ...	17			17			17			25		20		17		
n ...	(12)			(14)			(17)			(18)		(9)		(10)		
D ...	May 3			Oct. 4			May 5			Sept. 27		Apr. 23		Apr. 26		
Day	C.P.	O.M.	M.	C.P.	O.M.	M.	G.C.	O.M.	M.	G.C.	M.	C.P.	SPR.†	C.P.	O.M.	SPR.†
1	0	2	1	0	3	2	0	3	0	0	3	2	5	0	2	2
2	0	4	5	3	6	6	3	4	4	1	6	6	11	0	8	9
3	3	15	15	9	17	14	—	—	—	7	17	—	—	1	13	11
4	9	16	17	12	—	16	13	11	10	11	25	9	14	2	15	16
5	15	17	—	16	—	17	17	14	16	16	—	—	—	3	22	18
d	*7*	—	—	—	—	—	—	*8*	*6*	*7*	—	—	—	—	—	—

* *Beta vulgaris*, mangold, variety "yellow oil-berry" (*sárga olajbogyó*). Some capsules had more than one sprout.
† SPR. = sprinkled with 1·2 gm. of radioactive mud.

N = no. of seeds used; *n* = no. of experiment; *D* = date of commencement of experiment (day 0). S.C. = control in sand; C.P. = control on filter paper; G.C. = control in garden soil; O.M. = on filter paper over radioactive mud; M. = in radioactive mud. When the maximum number of germinated seeds (*N* or *N*−1) was not attained during 5 days, the number of the day upon which it was attained is given in italics opposite *d*.

In the case of the seeds laid directly on the radioactive mud, exhaustion of seed reserves is not an adequate explanation for the tendency to parallelism of final growth, of controls and treated seeds, and it is suggested that after an initial period of stimulation there was a repressive effect due to radiation. This suggestion is supported by the fact that poorer growth sometimes occurred with the larger amounts of mud. It is also possible that the mud (apart from its radioactivity) was an unkindly soil, so that two unfavourable factors operated cumulatively.

Table IIIA. *Shoot length.*

		Length of shoot (mm.) (average for all germinated seeds)									
Day of observation...		*2*	*3*	*4*	*5*	*6*	*7*	*8*	*10*	*11*	*13*
Triticum	C.P.	11	18	—	40	—	—	—	—	98	—
(3)	O.M.	14	36	—	65	—	—	—	—	160	—
	M.	15	37	—	70	—	—	—	—	150	—
Pisum	C.P.	—	2	5	10	18	—	29	45	—	55
(14)	O.M.	—	4	9	17	23	—	43	52	—	54
	M.	—	6	11	18	22	—	46	76	—	88
Phaseolus	G.C.	—	—	—	6	—	23	—	59	—	79
(17)	O.M.	—	—	—	5	—	16	—	38	—	74
	M.	—	—	—	4	—	12	—	43	—	73
*Beta**	C.P.	—	3	5	—	—	—	—	—	—	—
(10)	O.M.	—	9	17	—	—	—	—	—	—	—
	SPR.†	—	6	15	—	—	—	—	—	—	—

* † etc. Remarks as for Table III.

The information given in this table is supplemented by Text-fig. 5.

French beans (*Phaseolus*) and soya beans showed an apparently marked sensitiveness to irradiation, but in the case of the French beans the final results were not conclusive owing to proneness of these seeds to microbial invasion. Exposed both upon and above the mud, *Daucus* showed a slight acceleration of early growth; with the normally rapidly germinating *Raphanus* no difference in the speed of germination was detected under irradiation, which produced, however, a distinct increase in shoot length. A modification of the technique was employed in some experiments with mangold (*Beta*). The mangold "seeds" (capsules) were irradiated by sprinkling 1·2 gm. of the mud directly upon moist filter paper between the seeds. Whether irradiated thus directly with a minute amount of mud or with a dose of about 70 gm. through the usual interposed Petri dish and filter paper, the mangold seeds showed a stimulation of germination and growth as compared with the unirradiated controls; the stimulation was, however, perceptibly smaller in seeds lying amidst a sprinkling of mud, and exposed to no other radiation.

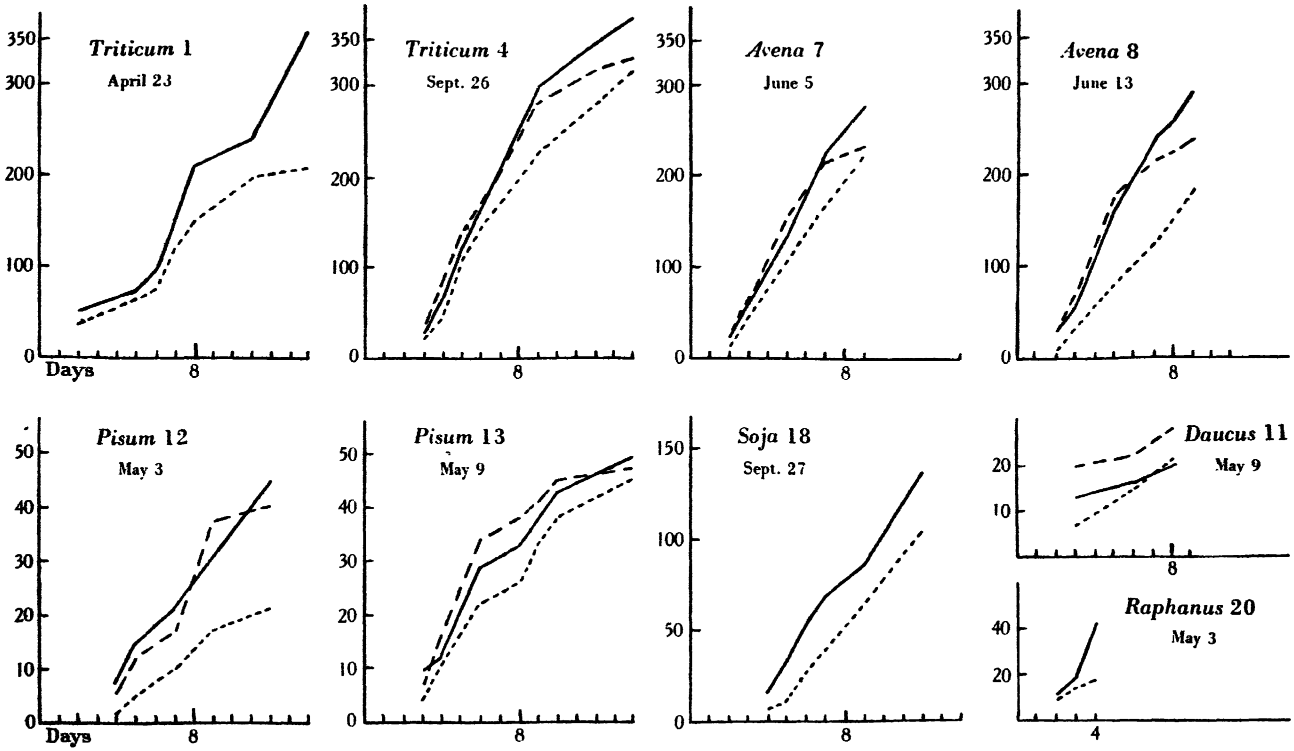

Fig. 5. Graphical representation of some results of direct and indirect exposure to radioactive mud. - - - - Controls: on filter paper (except Exp. 1, where the controls were in sand, and Exp. 18, where they were in garden soil). — — — Seedlings in a Petri dish over radioactive mud. —— Seedlings in radioactive mud. These typical results are additional to those given in Table III A.

Vertical heights represent shoot length in mm. (average values of twenty-five to forty seeds per experiment). Horizontally each mark represents 1 day, beginning with the day of sowing, recorded on the graph.

The observed differences in the sensitiveness of seeds under the conditions of this set of experiments are in fair agreement with those described in the preceding section as far as the effect of radioactive mud upon the early stages of vegetation is concerned (see Dognon, 1930; Stoklasa, Hruban, and Penkava, 1930). Thus, freshly harvested peas were found to be more sensitive to radiation operating during germination than were peas of a previous year's harvest. There was evidence, as in the previous experiments, that different strains of one species of seed (oats, wheat) were differently susceptible to irradiation. Further work requires to be done upon these seasonal and varietal differences.

Radiomorphoses (partial discoloration and other phenomena) were observed in the leaves of seedlings of mangolds, soya beans and peas, whenever these were germinated upon a quantity of mud exceeding 220 gm. On the other hand, radishes and carrots directly exposed to mud had darker leaves than the controls. Similar but much less marked phenomena occurred in these same five species when they germinated in Petri dishes above the radioactive mud. Plate II*a* shows a twisting of the leaves of wheat, observable even in presence of the smaller amounts of mud; the graminaceous plants displayed also an intertwining of their roots. The most characteristic and most frequent of these secondary phenomena of irradiated growth was the behaviour of the leguminous roots: instead of penetrating vertically into the mud, they remained on the surface up to a late stage of growth. This was undoubtedly a negatively geotropic effect such as Montet (1932) had observed under the influence of weak radioactivity.

It should be noted that the observations described in this section relate to growth under conditions of continuous diffuse irradiation, which must have different effects upon the various parts of the young seedling (Redfield and Bright, 1922). The susceptibility of the various parts may, moreover, be supposed to vary at different stages of growth, and one must take into consideration not merely the penetrating radiation but also the emanation and the degree of ionisation of the air (see Körnicke, 1904; Stoklasa, 1930; and Ingber, 1931).

(*b*) (ii) *The screening effect of interposed lead sheet.*

In order to show that the stimulative effects generally noted in the preceding section were chiefly due to radiation, three series of experiments were made by interposing a sheet of lead between the radioactive mud and oats, wheat and peas. Each series included ten experiments performed in May, June and July, in each case using thirty oats, thirty wheat and seventeen pea seeds. One Petri dish, containing the seeds placed upon moist filter paper, was placed over another empty or filled with about 65 gm. of radioactive mud, both with and without interposing a recently remelted sheet of lead about 4 mm. thick, covering the entire lower dish. This scheme would have demonstrated, *inter alia*, a proper radioactive effect of the lead, had there been any, but none was detected. A conspectus of some results is given in Table IV.

When the lead sheet was omitted the experiment served as an extension of those described in the preceding section, of which part of Table IV is therefore an amplification, the results of irradiation being similar. Whenever the lead was interposed between seeds and mud, the radiation was effectively cut off, so that no significant difference in germination or growth could be perceived between the seeds not irradiated and those placed over the lead sheet over mud. In some three cases, however, with the lead sheet in place, small and probably non-significant differences from the controls were observed (both positive and negative) for which figures are not given in detail. It is possible that phenomena of atmospheric electrical charge (cf. Vlés, 1930) were in some part responsible for those divergencies from the normal, since, when they were observed (May 18–28, 1933), the barograph registered marked fluctuations.

(*c*) *Effect of radiation upon seeds bathed in dyestuff solutions.*

The combined effect of irradiation and dyestuffs in solution was tried upon wheat. As a general rule comparison was directly made between seeds grown in dye solution, irradiated and not irradiated, in Petri dishes with a technique similar to that already used. An absolute control—that is, of seed neither dyed nor irradiated—was set up only once; for the most part growth of dyed seeds, irradiated and not, was compared with the controls of the preceding experiments.

The dyes used were: orange G (Grübler), methylene blue (Grübler), and neutral red (Merck), all dissolved in distilled water at concentrations of 1 in 10,000 and 2 in 10,000. Thirty seeds (placed upon filter paper in a Petri dish) were soaked in 5 gm. of the solution of which the effect was to be tested. The dish containing the seeds was placed over a Petri dish filled with radioactive mud, or even sand, or nothing, for the control. The whole was kept in the dark throughout, at temperatures from 18 to 25° C., in order that the degree of vital staining, if any, should be better perceptible through etiolation. Distilled water was added as evaporation proceeded.

Table IV.

Controls on filter paper.
Rad. =exposed upon glass above radioactive mud.
Pb. =exposed upon glass over lead sheet alone.
Pb.Rad. =exposed upon glass over radioactive mud with lead sheet interposed.

Species	No. of seeds		Average no. of germinated seeds					Mean shoot length (mm.)					Mean root length (mm.)					
Day of observation		...	*1*	*2*	*3*	*4*	*5*	*2*	*3*	*4*	*5*	*7*	*2*	*3*	*4*	*5*	*6*	*7*
Triticum	30	Control	7·5	26	29	29·5	29·5	3	8·5	25·5	33	60	10·5	22	34	35	—	—
		Rad.	15	27·5	29·5	29·5	29·5	5·5	20	32·5	50	80	11·5	31	44	63·5	—	—
Avena	30	Control	8	29	29·5	29	29	0·5	5·5	24	39	68	8·5	22	33	35	—	—
		Rad.	13	26·5	26·5	27	27	9	16	39·5	50	82	11·5	25·5	36	55	—	—
Pisum	17	Control	0·5	2	13·5	15	15	0	0	4	10·5	18	0	9·5	16	20	30	27
		Rad.	1	8·5	14	16	16	0	0·5	10·5	16	29	7·5	18·5	27·5	30	30	35
Triticum	30	Control	8	28	29	29	30	2·5	13	26·5	41·5	60	9	28	44	55	—	—
		Pb.Rad.	10	27	30	30	30	3	13	26·5	40·5	53	10·5	29	46	54	—	—
Avena	30	Control	12	27	28	28	29	+	9·5	26·5	47	68	9	27	42	51	—	—
		Pb.Rad.	11	24	28	28·5	29	+	9	26	42	68	11	28	41	49	—	—
Pisum	17	Control	1·5	8	16	16	16	0	3	12	17	16	2	9	25	31	35	27
		Pb.Rad.	1·5	8	15	16	15·5	0	3	12	17	25	2	10	23	31	33	38
Triticum	30	Control	5·5	25·5	29·5	29	29	1	8	25	41	60	5·5	26	41	—	—	—
		Pb.	4	27	29·5	29·5	29·5	0·5	7	24	42	62	4·5	25	42	—	—	—
Avena	30	Control	4	25	27	27	28·5	0	4·5	21	48	68	6	24	38	—	—	—
		Pb.	2·5	26	28	29	29·5	0	3·5	22·5	43	69	5	24	39	—	—	—
Pisum	17	Control	2·5	10	16	16	16·5	0	3·5	14	21	16	4	11	27	33	30	27
		Pb.	1	8	14	16	16	0	3	13	17·5	15	3	13	27	33	29	27

Average results are given in Text-fig. 6. Both concentrations of methylene blue and neutral red showed a slight stimulation of germination, and of growth, in the irradiated dyed seeds as compared with those dyed but not irradiated. This statement referring to the relative values is not invalidated by absolute values being greater, the higher the temperature.

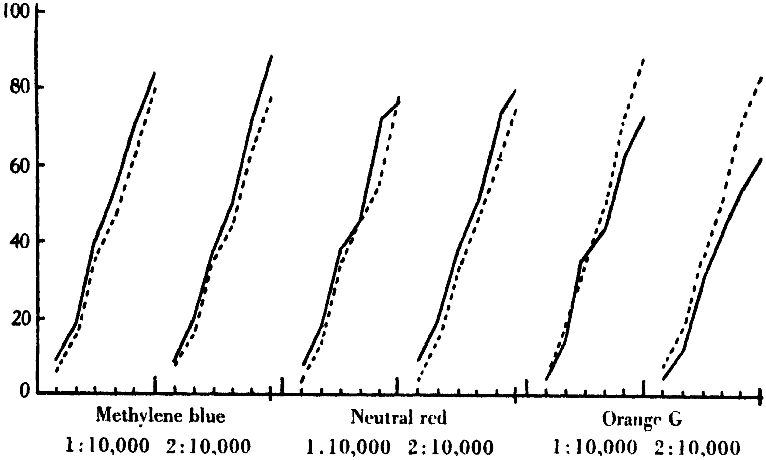

Fig. 6. Effects of radiation upon wheat seeds bathed in dyestuff solutions. Average values of shoot length for thirty seedlings per experiment. The horizontal marks indicate days from the commencement of observations. - - - - Dyed seeds not irradiated. —— Dyed seeds irradiated.

Differences were most marked on the third day at 25° C., and on the fourth day at 18° C. Still more interesting, perhaps, is the fact that orange G in similar circumstances and more especially in the 2 in 10,000 concentration, brought about a small but indubitable repression of growth in the irradiated seeds, although its effect upon the seeds not irradiated was to bring about a slight enhancement of rate of growth: in this last particular, orange G resembled the other two dyes.

Histological examination of the dyed seedlings showed no appreciable difference between those irradiated and those which had not been placed over mud.

(*d*) *Soil experiments with radioactive mud in pots.*

French beans (Hungarian pearl bean, *Phaseolus vulgaris*). Twenty-four pots of a capacity of about 1500 gm. of soil were divided into four series, each of six pots. Thirty seeds were sown per pot. Series A (control) was filled with garden soil (for analysis, see Appendix). Series B contained a similar weight of a mixture of one-third of radioactive mud and two-thirds garden soil. Series C contained about 1 kg. of garden soil, in which was almost buried a glass cylinder, the rim of which projected slightly, to prevent mixing of the two soils.

Seeds were sown in the outer (garden soil) portion. Series D contained 1500 gm. of radioactive mud. (All weights are per pot.) Fertiliser of the composition (per cent. by weight) 40 per cent. sodium nitrate, 30 per cent. ammonium sulphate and 30 per cent. di-hydrogen potassium phosphate was incorporated with dry soils and soil mixtures at the rate of 2 gm. per 1500 gm. soil. Watering was afterwards done as necessary.

Soya beans. The technique was similar but for two divergencies. In series A, B and D a total of only 1 kg. of soil was put into each pot. In series C^1 the glass cylinder was replaced by a completely paraffined pot; this was filled with about 1 kg. of garden soil in which the seeds were sown. The pot was placed inside a larger one and the intervening space filled with about 1500 gm. of mud. The positions of mud and soil were thus reversed as compared with series C preceding. Eighteen seeds were sown in each pot.

Wheat. Only two series were set up, each in fully galvanised iron cylinders 80 cm. high and 30 cm. diameter, with a capacity of 55 kg. of soil. Series A was filled with garden soil, and series B with the mixture of one-third mud and two-thirds garden soil. The same fertiliser mixture was used as for the preceding experiments, but at the rate of 2 gm. per 3 kg. of soil.

In all experiments seeds were sown on the surface. The pots were kept in a greenhouse, and frequently moved at random to diminish positional effects.

Results. A glance at Table V will be sufficient to show that the neat mud (series D) appreciably stimulated germination and early growth of both kinds of beans. The effects of the mixture of mud and garden soil (series B) were not limited to the initial stages of growth, but were markedly beneficial throughout the period of observation. The arrangement whereby the seeds were in proximity to, but not in contact with, the mud (series C and C^1) gave effects intermediate between those of series D and B. Other similar experiments, not here recorded in detail, with peas and mangolds, led to the same conclusions.

With wheat the first effects of the diluted mud were not favourable, but later observations showed an earlier flowering of the plants of series B.

It should be borne in mind that in such experiments in pots, with

Table V. *Results of experiments with soil in pots: averages of six pots per series.*

	Series	No. of seeds per pot	No. of germinated seeds							Shoot length (mm.)				
Date of observation: May 1933 ...		...	*2*	*4*	*6*	*9*	*11*	*17*	*25*	*6*	*9*	*11*	*17*	*25*
Phaseolus	A	30	3	9	18	25	26	27	26	31	60	75	80	141
	B	30	6	15	24	27	27	27	25	40	76	80	110	163
	C	30	2	13	25	29	29	29	26	42	72	85	100	136
	D	30	5	17	25	28	29	30	28	45	80	85	110	149
May-June 1933		...	*9*	*11*	*16*	*1*	*12*	*24*	—	*11*	*16*	*1*	*12*	*24*
Soja	A	18	5	15	16	17	17	17	—	3	29	103	144	213
	B	18	8	18	18	18	18	18	—	8	38	143	216	291
	C^1	18	4	14	17	17	18	18	—	7	34	126	149	225
	D	18	10	18	18	18	18	18	—	7	39	106	139	185
June 1933 ...		...	*18*	*21*	*22*	*23*	*26*	—	—	*21*	*22*	*23*	*26*	*30*
Triticum	A	30	11	26	28	28	28	—	—	23	50	90	142	161
	B	30	7	20	30	30	30	—	—	20	55	90	150	174

A = seeds in garden soil.
B = seeds in mixture of garden soil and radioactive mud.
C and C^1 = seeds in garden soil in proximity to mud.
D = seeds in mud.

seeds in immediate contact with soil, they were exposed to both radiation and emanation in a much more direct way than in those experiments carried out with the seeds placed in a Petri dish lying above another containing mud. Radiomorphosis was frequently observed in the pot-experiment plants.

III. DISCUSSION.

These results show that both stimulating and depressant effects upon early growth of plants are exerted by the radioactive mud employed, in amounts containing from 8×10^{-8} mg. Ra (powdered mud dusted on filter paper) up to $2{\cdot}3 \times 10^{-3}$ mg. Ra (in remote radiation experiments). The range is thus very wide, but on account of the variation in experimental conditions, no relationship can be deduced between Ra content and the effects noted[1]. It is nevertheless noteworthy that an appreciable stimulation was found with minute doses of mud, and the approximate limits of Ra content thus indicated are in fair agreement with those found by other workers using other techniques. Stoklasa (1912) who used a radioactive mineral, estimated the maximum tolerated dose of Ra not to be more than $3{\cdot}4 \times 10^{-3}$ mg.; Molisch (1912) found that $6{\cdot}2 \times 10^{-5}$ mg. of emanation harmed his plants. Stoklasa (1920)—to mention only his emanation-chamber experiments—required 0·08–0·009 M.-E. of emanation per litre of air to initiate stimulation of plant growth. Lazarus-Barlow and Beckton (1913) obtained stimulation with a minimum dose of 5×10^{-7} mg. Ra.

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[1] It is theoretically possible, if the graph of stimulation against dosage is convex, that the same degree of stimulation might be obtained by two very different doses, one minimal and the other just less than that which produces a harmful effect.

By methods more comparable with those described in this paper, Mezzadroli and Vareton (1931) who employed an Italian radioactive soil, observed a marked stimulation of growth after dusting 1 gm. of soil, of Ra content not exceeding $4{\cdot}2 \times 10^{-6}$ mg. per kg., over 10 sq. cm. of ordinary agricultural soil. This amount is about one-nineteenth of the smallest dose used in the present work. Jirkovsky (1931) has stressed the importance of natural radioactivity of cultivated soils, and has suggested that there is a direct relationship between their radioactivity and their fertility.

Should radioactive material, such as the natural mud used in this work, be sought to be employed as an addition to cultivated soil (Stoklasa (1928)), there would seem to be little fear of overdosage if reasonable precautions be taken. The range of permissible dosage appears wide. More work will be needed before the ancillary problem of determining the most economical dosage can be solved.

IV. SUMMARY.

Stimulatory effects upon germination and growth were observed in a very wide range of dosages, upon exposing seeds and seedlings to the radiation from a radioactive mud of Hungarian origin. Emanation was not excluded, but interposed lead sheet practically abolished the significant differences. Stimulation was observed whether the seeds were placed directly in contact with, or at a little distance from, all but the largest amounts of mud. Treatment of seeds, before sowing, with the radiation from large amounts of mud for periods exceeding about 48 hours was harmful to germination and to growth; this harmful effect was more marked when the seeds were moistened.

The degree and the kind of stimulation varied with the kind of seed and its history. Wheat seeds treated with vital stains showed responses to irradiation which appeared to depend upon the stain.

V. ACKNOWLEDGMENTS.

The author is glad to express his thanks to Prof. G. Entz, Director of the Hungarian Biological Research Institute, Tihany, Hungary, for giving facilities for the work to be done there: to the Directors of the Héviz Baths Company, for supplying the radioactive mud with which the experiments were performed at Tihany, and to Dr K. Kunffy for supplying some seeds grown on his land. The author's thanks are also due to Dr H. W. Lissmann, formerly of Tihany, for much assistance in the preparation of a German translation of the manuscript, and its numerous tables and diagrams; and to Dr Hugh Nicol, of Rothamsted Experimental Station, for preparing this shorter English version and for seeing it through the press.

VI. APPENDIX.

Chemical and physical features of the radioactive mud and garden soil used (Prof. A. A. J. de 'Sigmond):

	Mud (from Héviz)	Garden soil (from Tihany)
*p*H in aqueous solution	7·46	7·92
*p*H in KCl solution	7·28	7·58
Percentage of:		
CaCO$_3$	46·10	17·71
N	1·01	0·286
Humus	35·25	5·82
Available P$_2$O$_5$ (mg.)	131·5	37·5
Available K$_2$O (mg.)	26·1	29·7

The last two determinations were made and expressed by 'Sigmond's methods.

The garden soil had been prepared by mixing these constituents, expressed as approximate percentages of the total: sand, 17·5; leaf mould, 23·5; 3-year-old compost, 11·8; one-year-old compost, 34·5; loam, 11·8.

The radioactive mud was derived from a lake at Héviz, of greatest depth 36·5 m. An analysis of the water of the lake has been given by Emszt (1932): total solids 0·067 per cent., principally calcium and magnesium bicarbonates.

No absolute determination of the radium content of the mud was made directly, but an evaluation was reached indirectly as follows: according to Dr A. Hajdu, the Héviz mud is 2·17 times as radioactive as is the *fango* of Battaglia, Italy (figure supplied by courtesy of Prof. G. Weszelszky); the published analyses of Nadig (1925, 1930) and of Nussberger (1926) ascribe to the radioactive mud of Val Sinestra, Switzerland, a radioactivity some ninety times that of the Battaglia mud, and a radium content of 0·0028 mg. Ra per kg. of mud. Hence the Héviz mud used had a mean radium content of 0·000067 mg. Ra per kg.

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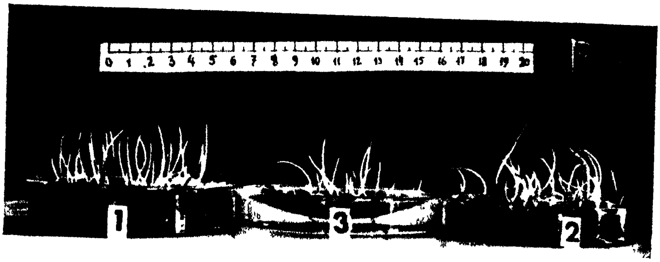
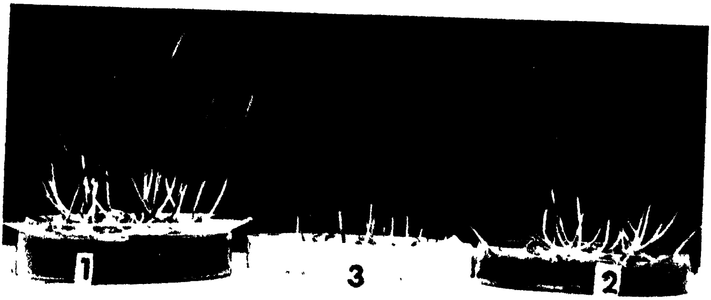


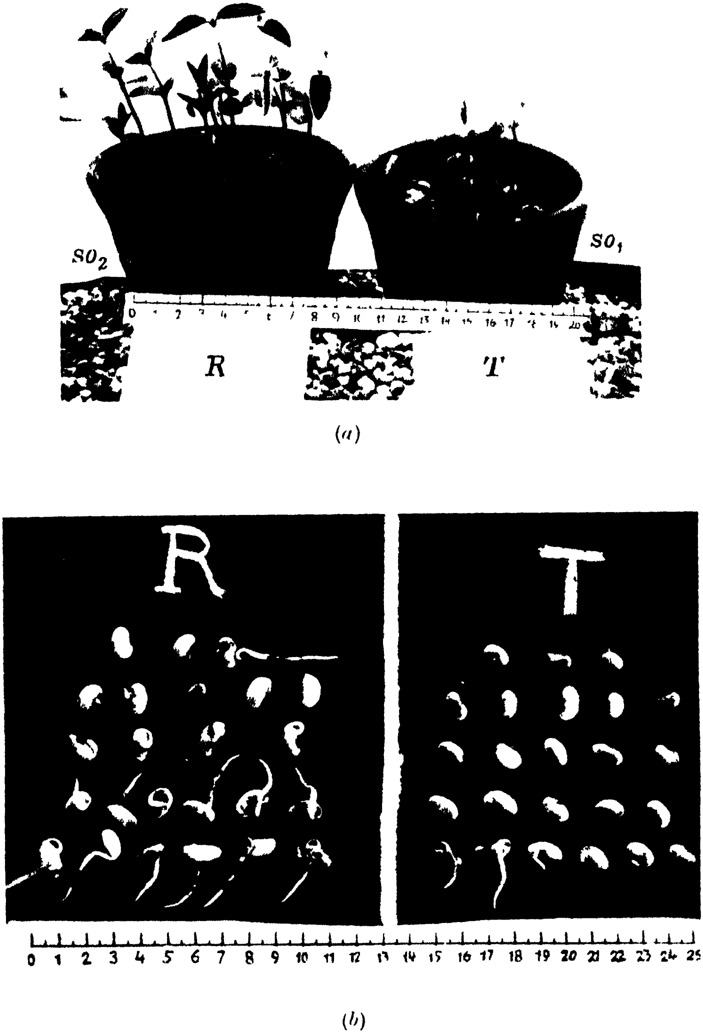
(*a*)



(*b*)

a. Effects of direct and indirect exposure of wheat seeds to radioactive mud. Exp. 1, Table III and Text-fig. 5. (1) Seedlings on clock glass over radioactive mud. (2) Seedlings in radioactive mud. (3) Control seedlings on filter paper.

b. Effects of direct and indirect exposure of oat seeds to radioactive mud. Exp. 6, not otherwise represented in this paper. (1) Seedlings on clock glass over radioactive mud. (2) Seedlings in radioactive mud. (3) Control seedlings on filter paper.



Effects of direct exposure of soya beans to radioactive mud. Exp. 19, not otherwise represented in this paper. (*a*) Left, plants about 15 days old in radioactive mud; *R*, right, plants of the same age in garden soil, *T*. (*b*) Left, seeds after 3 days in radioactive mud; the majority have germinated, *R*. Right, control seeds after 3 days on moist filter paper; few have germinated, *T*.

THE VITAMIN A REQUIREMENT OF SWINE.

By GEORGE DUNLOP, *Ministry of Agriculture Research Scholar.*
(*From the Animal Nutrition Research Institute,
University of Cambridge.*)

(With One Text-figure.)

SINCE the publication of my previous work on avitaminosis A in swine (5) the occurrence of the disease has been reported at two other research institutes (Reading and Rothamsted) in this country (9, 11). The classical symptoms of the deficiency developed in both instances on rations which must often be used in practice. In our own experiments we had been able to show that the time-honoured mixtures of (1) barley meal, middlings and fish meal and (2) cereals, soya-bean meal and minerals did not satisfy the requirement of growing swine for vitamin A. While the conditions which give rise to the disease and the doses of therapeutic reagents necessary for its cure are now adequately known, there is no exact information as to the types and amounts of foodstuffs in a ration which should be used for its prevention. The present paper reports experiments which were carried out partly with the object of supplying an answer to this question and partly to obtain further information on the actual vitamin A requirement of swine for growth to bacon weights.

EXPERIMENTAL.

The animals used were four complete litters (thirty piglings in all) obtained at 8 weeks old from a local farm. The weights ranged at that time from 27 to 46 lb. Mr E. A. Porter, who was in charge of the animals, observes in the record sheets that all were in good condition on arrival.

The allotment of these animals to the various treatments was made by withdrawing their corresponding ear numbers from a hat. The litters from which they were drawn are given below. It is possible that the arrangement might have been bettered, but the non-arrival of a consignment of rice meal to be included in two of the rations imposed certain restrictions on the lay-out and ruled out a complete randomisation.

Treatment	Animals from litter	Treatment	Animals from litter
1	A, B, C	6	C, D, D
2	A, B, C	7	A, B, C
3	A, B, C	8	A, C, D
4	A, B, C	9	A, B, C
5	C, D, D	10	A, B, D

The rations used were as follows:

RATION 1	
Barley meal	84 parts
Extracted soya-bean meal	16 ,,

RATION 2	
Barley meal	84 parts
Fish meal	16 ,,

RATION 3	
Barley meal	60 parts
Weatings	24 ,,
Extracted soya-bean meal	16 ,,

RATION 4	
Barley meal	24 parts
Weatings	60 ,,
Extracted soya-bean meal	16 ,,

RATION 5	
Barley meal	60 parts
Rice meal	24 ,,
Extracted soya-bean meal	16 ,,

RATION 6	
Rice meal	84 parts
Extracted soya-bean meal	16 ,,

RATION 7	
Barley meal	60 parts
Maize meal	24 ,,
Extracted soya-bean meal	16 ,,

RATION 8	
Barley meal	24 parts
Maize meal	60 ,,
Extracted soya-bean meal	16 ,,

RATION 9	
Barley meal	60 parts
Weatings	24 ,,
Extracted soya-bean meal	14 ,,
Alfalfa meal	2 ,,

RATION 10	
Barley meal	60 parts
Weatings	24 ,,
Extracted soya-bean meal	10 ,,
Alfalfa meal	6 ,,

To every 100 lb. of rations 1, 3, 4, 5, 9 and 10 were added 1½ lb. ground limestone, ½ lb. common salt and 1 oz. ferric oxide.

To every 100 lb. of rations 6 and 7 were added 1 lb. of ground limestone, ½ lb. steamed bone flour, ½ lb. common salt and 1 oz. ferric oxide.

To every 100 lb. of ration 8 were added ½ lb. ground limestone, 1½ lb. steamed bone flour, ½ lb. common salt and 1 oz. ferric oxide.

To ration 2 no addition was made.

The addition of steamed bone flour to rations 6, 7 and 8 was found to be necessary, since maize meal (1) and to a certain extent rice meal contain less phosphorus than the other cereals used. The resulting Ca : P levels and ratios all lay well within the anti-rachitogenic area of the chart indicating the requirement of swine for these elements (6). Thus rachitic conditions were not a complicating factor in the experiments.

The carotene content of the foodstuffs was again determined by a method previously described ((5), p. 443). The values found are given in Table I.

Table I. *Carotene content of the foodstuffs used in the experimental rations.*

Foodstuff	γ carotene per gm.	mg. per lb.	mg. per 100 lb.
Alfalfa meal	11·40	5·13	513·34
Barley meal	0·76	0·34	34·38
Fish meal	0·09	0·04	4·20
Maize meal	2·89	1·32	131·77
Rice meal	0·60	0·27	27·03
Extracted soya-bean meal	0·46	0·21	20·92
Weatings	0·52	0·24	23·65

The technique used in feeding the animals during the experiment has been reported in a previous communication in which the plane of nutrition and the method adopted in calculating the results for growth rate are also given (4).

RESULTS.

Growth.

In considering the growth of the animals (as determined by their live-weight increase) various aspects of the phenomenon must be presented to give a clear picture of the effects of the rations. In the first place, the actual growth rates obtained on the various diets, when these were consumed by the animals in similar amounts, will give some idea as to their suitability for live-weight increase and economy of gain. In other words, these observations will show the comparative net energy value of the rations. While certain animals refused food during the early stages of the experiment most of them reached 120 lb. before doing so. The growth rates of these latter animals are therefore comparable up to this live weight and the pertinent data of these "survivors" are given in Table II.

As the animals were not killed off until some time afterwards, the results given here must remain uncorrected for differences in the amounts of fat and protein which the animals laid down up to this time (7). They are sufficiently accurate for the present study, however, as where duplicate or triplicate determinations have been obtained on the same ration the variation is not great.

Table II. *Live-weights of animals (in lb.) 8 weeks after reaching* 50 *lb.*

	Pig from litter				
Ration	A	B	C	D	Average
1	0	121	0	—	121
2	123	122	119	—	121
3	112	0	0	—	112
4	Died	0	Died	—	
5	—	—	Died	0, 0	
6	—	—	0	0, 0	
7	115	117	0	—	116
8	0	—	0	121	121
9	Died	110	112	—	111
10	114	111	—	116	114

0 = animal assumed lower plane of nutrition than those for which figures are given.

While the number of animals which did remain on the same fixed plane of nutrition are too few to draw definite conclusions as between one ration and another, the results do show the inferiority of weatings compared with whole cereals in relation to their net energy values. The rations which included weatings—25 per cent. in each case—(Nos. 3, 9 and 10) produced slower growth rates than those in which the weatings were replaced by cereals (Nos. 1, 2, 7 and 8). These results therefore do not support the theories advanced by Fisher and Shaw (8) concerning the feeding value of straight-run middlings (weatings).

While the comparative net energy values show that all rations provided sufficient digestible carbohydrate and protein for normal live-weight gains they do not really concern us here. They give no indication of the suitability of the ration for supplying the animal with sufficient vitamin A for growth purposes. The value of the rations in this respect is best demonstrated by considering the live weights at which growth ceased and a decline in live weight commenced. These are shown in Table III and graphically in Fig. 1.

Table III. *Live-weights of animals at which further growth in weight ceased.*

	Animal from litter			
Ration	A	B	C	D
1	88	129	79	—
2	[194]	[182]	162	—
3	118	88	72	—
4	66	91	73	—
5	—	—	70	124, 89
6	—	—	59	44, 44
7	[170]	[193]	[160]	—
8	[164]	—	[189]	[152]
9	69	130	[143]	—
10	[160]	[201]	—	[162]

[ ] animal still growing normally—slaughtered and liver analysed for concentration of vitamin A.

When the live weight of the animals began to decline certain of them received daily supplements (5 ml.) of a vitamin A concentrate dissolved in arachis oil. In every case this treatment proved successful in restoring normal growth. To one animal (on ration 1) the oil, in which the concentrate was dissolved, was fed alone. The live weight continued to remain stationary during the four-week period of this treatment. On feeding the concentrate plus oil at this stage normal growth and food consumption were restored within one week.

When the animals either died or were killed off, the amount of vitamin A in the livers was estimated by Davies's method (3). Duplicate samples of the livers were sent to Mr A. L. Bacharach of the Glaxo Research Laboratory, who kindly confirmed our results by the colour

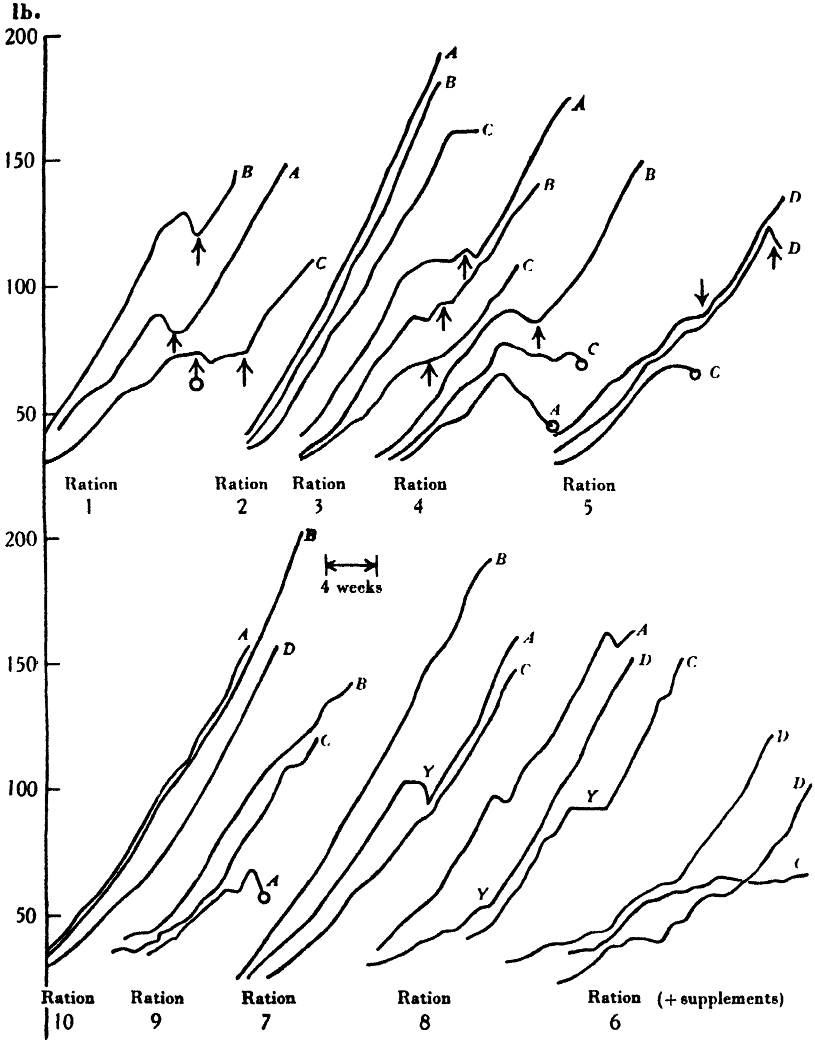


Fig. 1. Weight-growth curves of the experimental animals. The figure shows that all rations, except No. 10, were deficient in at least one vitamin. Ration 10, however, may be considered deficient in net energy value for maximum growth and economy of gains (cf. growth rates of normal animals (A and B) ration 2). The immediate response to the vitamin supplements is most noticeable.

♂ arachis oil, 5 ml. per day.
↓ vitamin A + arachis oil (vitamin concentrate), 5 ml. per day.
Y 1 oz. yeast per day.
0 died.

The letters (A, B, C and D) refer to the litters from which the animals were taken.

test and by the spectroscopic method. The results of the analysis by Davies's method are given in Table IV.

Table IV. *Vitamin A content of livers expressed as blue units (Moore's) per gm. fresh liver.*

Ration no.	Ration alone Animal from litter				Ration + vitamin A therapy Animal from litter			
	A	B	C	D	A	B	C	D
1	—	—	—	—	2	<2	8	—
2	<2	Trace	Nil	—	—	—	—	—
3	—	—	—	—	<2	4	<2	—
4	Nil*	—	Nil*	—	—	6	—	—
5	—	—	Nil*	—	—		—	2, <2
6	—	—	—	—	—	—	<2	2, 25
7	6	6	<2	—	—		—	—
8	25	—	25	25	—	—	—	—
9	Nil*	Nil	Nil	—	—	—	—	—
10	3	3	—	2	—	—	—	—

* Animal died.

DETAILED DISCUSSION OF THE RATIONS.

Ration 1: barley meal 84, extracted soya-bean meal 16 + mineral mixture. The typical symptoms of avitaminosis A—swaying gait, fits, paralysis, distorted necks and partial blindness—developed in all three animals. The symptoms were corrected in each case by daily supplements of 5 ml. of the vitamin solution, complete recovery taking place within 3 weeks. The animal from litter B was consuming only 1·5 lb. of the ration per day and was in a very weakened state when the concentrate was first fed. After 17 days it had returned to full feed (5·15 lb.), the symptoms had disappeared and it had again become an apparently normal animal. It was killed off 2 days later, having gained 26 lb. in the 19 days. Only a trace of vitamin A (Bacharach, 0·1 I.U. per gm.) was present in the liver, showing that the requirements of the animal for this amazing recovery had just been satisfied. The animal from litter A received the vitamin therapy for 7 weeks (gaining 53 lb.) and had stored 2 units per gm. in its liver. The potency of the arachis oil *per se* was tested on the litter C animal. At the end of 4 weeks no gain in live weight had been made and the symptoms of the disease had become more marked. On changing over at this stage to the vitamin concentrate (vitamin A + arachis oil), the animal gained 30 lb. in 5 weeks and was found to have stored 8 B.U. per gram in its liver.

Ration 2: barley meal 84, fish meal 16. The net energy value of this ration was superior to all others during the period in which this property of the diets was tested. Two animals in fact increased in weight from 40 lb. to bacon weight (190 lb.) in 15 weeks with an economy of gain of 2·98 and 3·01 lb. food (2·7 lb. dry matter approx.) per lb. live-weight gain respectively. When 162 lb. live weight the third animal developed leg weakness and its live weight remained stationary for a fortnight. It was then slaughtered and the liver was found to be devoid of vitamin A. The results also show that the reserves of the first two animals (Bacharach, 0·3 and 0·2 I.U. per gm.) were almost exhausted when bacon weights were reached. This finding is in good agreement with the conclusions arrived at in our previous study (5), namely that the standard barley meal, weatings, fish-meal ration does not meet the vitamin A requirement of swine for normal growth.

Ration 3: barley meal 60, weatings 24, extracted soya-bean meal 16 + minerals. The animals had ceased growth and had all but succumbed after 12 weeks of this dietary. Supplements of vitamin A brought about complete recovery within 1 month. The recovery growth rates were 63, 56 and 40 lb. all in 7 weeks, but in only one case was the storage of the added vitamin in the liver appreciable.

Ration 4: barley meal 24, weatings 60, soya-bean meal 16 + minerals. The effect of increasing further the weatings portion of the ration proved even more disastrous. Two animals died of necrotic enteritis. One of these animals had been declining in weight for over a month before vitamin A therapy was commenced. It died, however, 2 days later. The third animal showed a growth rate of 52 lb. in 7 weeks after receiving the supplement of which it had stored 6 B.U. per gm. in its liver at time of slaughter.

Ration 5: barley meal 60, rice meal 24, soya-bean meal 16 + minerals. All the animals had exhausted their initial reserve before reaching 90 lb. One died at 70 lb., death being again due to enteritis. The remaining two animals received doses of the vitamin concentrate, one making a gain of 46 lb. in 6 weeks. The other showed a gain of only 5 lb. in a fortnight when it was 80 lb. live weight, but appeared to make a spontaneous recovery for some time at least. At 124 lb. it suddenly collapsed and the supplement was fed for 6 days before it was finally killed off. The storage during this short period was 2 B.U. per gm. (Bacharach, 2·5 I.U. per gm.), but outward signs of recovery were not apparent.

Ration 6: rice meal 84, soya-bean meal 16 + minerals. As was expected, this ration proved to be deficient in more factors than one, and the animals scoured continuously. An animal from litter D eventually made normal growth with the following supplements: 10 per cent. blood meal, 30 gm. yeast and ½ lb. green food per day. At slaughter it showed the comparatively high concentration of 25 B.U. per gm. vitamin A in its liver (Bacharach, 18 I.U. per gm.). Of the remaining two, one showed paralytic symptoms before receiving supplements of green food, while both showed low reserves at slaughter—<2 B.U. and 2 B.U. per gm. (Bacharach, 1·0 and 3·5 I.U. per gm.)

Ration 7: barley meal 60, maize meal 24, soya-bean meal 16 + minerals. Two of the animals grew normally for the greater part of the trial (one experienced a setback for 4 weeks when 100 lb. live weight). The blue values obtained on analysis of the liver samples were similar—6 B.U. per gm. (Bacharach, 10 and 11 I.U. per gm. respectively). The question arises as to whether these figures represent storage of an excess of vitamin A in the ration for the requirements of the animals or whether the ration was slightly subminimal in vitamin A, the amount of the vitamin in the liver being now less than it was at the beginning of the experiment. While neither alternative can be proved, we incline to accept the latter view which is supported by the experimental records of the remaining animal. After growing normally for 6 weeks this animal developed a staggering gait which, however, disappeared 3 days later. It was thereafter subject to repeated scouring attacks, and the growth rate and food consumption were only normal for about 2 weeks at a time. At slaughter analysis of the liver showed <2 B.U. per gm. (Bacharach, $<0{\cdot}2$ I.U. per gm.), which is sufficient evidence that the requirements of the animal for the vitamin were not being adequately met.

Ration 8: maize meal 60, barley meal 24, extracted soya-bean meal 16 + minerals. Bethke, Edgington and Kick (1) in their studies on the Ca, P and vitamin D requirement of swine used a basal ration of 84 per cent. yellow-maize meal and 16 per cent. soya-bean meal + salt. When a mineral mixture was added containing the correct amounts of Ca and P good growth was obtained. It is reasonable to suppose that the substitution of part of the maize with barley would give better results, but this was not our experience. A plateau in the growth curves occurred in the case of two animals after they had received the ration for 11 weeks. Now various workers have shown that somewhat over 50 per cent. of cereals in the ration is necessary to satisfy the requirements of rats for vitamin B_2. Maize, however, is grossly deficient in this factor. It appeared, therefore, that suitable supplements of this vitamin to the diet should exert some beneficial effect on the growth rate. This indeed proved to be the case. 1 oz. of brewers' yeast (about 1·5 per cent. of the total diet) produced an almost instantaneous resumption of normal growth which continued till the end of the experiment, 8 weeks later. In the case of the third animal the plateau occurred after 17 weeks and continued for a further 3 weeks when it was killed off without therapeutic treatment being attempted.

In each animal the concentration of vitamin A in the liver at slaughter was 25 B.U. per gm. (Bacharach, 24, 22 and 20 I.U. per gm.), which may be considered to represent an actual storage of an excess in the diet. It is noteworthy, however, that in satisfying the requirements of the animals for one vitamin we appear to have produced a deficiency of another. The construction and proper "balancing" of a ration for swine appears to be a much more difficult process than was formerly supposed.

While we are unaware of any previous record in the literature of swine suffering from a deficiency of vitamin B_2, Blisset and Golding(2) have already reported circumstances under which their animals developed a condition of avitaminosis B_1.

The only animal on this ration for which a proper record could be obtained for evaluating the net energy value of the diet was that from litter D. Throughout this period it received the yeast supplement. From inspection the ration may be considered to have a greater energy content than any other. Although the figure obtained does not support this observation it was really surprising to us that the animal made such a satisfactory gain in weight. Mr E. A. Porter observes that this pig "spends quite a lot of time walking in a circle round the pen (which is only 10 by 9 ft.), especially during the morning". The walk sometimes developed into a lively canter and was even resumed after an attendant had brought the animal to a standstill. Whether these were symptoms of some other pathological or deficiency condition we do not know, but the maintenance requirement of the animal must certainly have been greatly increased by these persistent and periodic peripatetics.

In general our experience with maize in the rations of swine agrees with observations of its effects on other animals. Much trouble is experienced in colonies of rats at research institutes where maize forms a large part of the stock diet, while in man its inclusion in the diet in any great amount is correlated with the incidence of pellagra in the populace.

Ration 9: barley meal 60, weatlings 24, extracted soya-bean meal 14, alfalfa meal 2 + minerals. One animal died suddenly after being 7 weeks on this diet. Post-mortem examination showed generalised enteritis of the intestines, while, somewhat unexpectedly, analysis of the liver showed that the body reserves of vitamin A were nil. The animal had been feeding normally up to a week previously when a staggering gait quickly developed and the live weight decreased.

Symptoms of avitaminosis A did not develop in the remaining two animals till the end of the experiment when they had been 20 weeks on the diet. They were killed off shortly afterwards when no reserves of vitamin A could be found in the liver of either animal (Bacharach, trace and 1·5 I.U. per gm.).

The practice in America of adding 1–1½ per cent. of alfalfa meal to the diet to satisfy the vitamin A requirement of swine is not applicable to conditions in Britain. As we have previously pointed out ((5), p. 453), alfalfa meal is a variable product in this country where the different methods adopted in curing the crop lead to wide fluctuations in its vitamin A potency.

Ration 10: barley meal 60, weatings 24, extracted soya-bean meal 10, alfalfa meal 6 + minerals may be regarded as the positive control ration in the experiment. Its high fibre content was perhaps the cause of the low food consumption and the slow growth rate of the animals in the early stages but, after they had reached 55 lb. live weight, all grew normally to bacon weights without exhibiting any untoward symptoms.

At slaughter, however, the vitamin reserves of the animals were at a low level, being of the same order as the normal animals on ration 7. One is forced to the conclusion that had the alfalfa meal used been of an even more inferior quality, the animals would have shown the symptoms of avitaminosis A which developed in the other groups.

PRACTICAL CONSIDERATIONS.

Of the ten rations used in this experiment seven have been proved to be deficient in vitamin A, two contained minimal or just subminimal amounts, while one appeared to permit storage. These rations, or, at least, slight modifications of them, are commonly used under practical conditions, and the conclusion that many animals in this country must be subject to various syndromes consequent on a deficiency of vitamin A in the diet, appears justified.

While none of the nine animals which developed the symptoms of avitaminosis A in our previous experiments (5) died, four succumbed on this occasion before therapeutic treatment was commenced. The conditions, however, under which the experiments were conducted were not similar in the two instances. On this occasion they may be said to approximate more nearly to conditions in farm practice. When the present experiment was undertaken the usual half-yearly disinfection of the pens and premises was not carried out on account of the premature arrival of the experimental animals. The "odour of pigs" was in evidence throughout the experiment, and owing to a prolonged illness of an assistant the hygienic conditions were not of the best. It is true that the faeces were removed daily from each pen, but on the other hand no attempt was made to wash down the feeding crates which in previous experiments had taken place at fortnightly intervals. Partly on account of this and partly on account of the origin of the animals the risk of infection was increased, with the result that some animals succumbed after the body resistance had been sufficiently lowered. It is noteworthy at least that there were no fatalities on the three rations providing appreciable amounts of vitamin A, while all the animals which died were on deficient rations and had depleted their body stores of the vitamin.

Golding and Foot (9) have shown that about 2 lb. of green food are necessary to meet the vitamin A requirement of swine, and where pigs are at pasture for some time after weaning they will normally store sufficient of the vitamin to tide them over to bacon weights when brought indoors and placed on deficient rations. The consumption of green food, however, slows up the rate of gain, and the practice has been discontinued where a quick turnover of stock is the policy, in which case the diet is restricted to meals with a high net energy value. Maize meal and alfalfa meal are then the only two foods commonly used under these conditions for supplying the animals with vitamin A—except where cod-liver oil is fed as a supplement to the ration. Both these meals, however, have been shown to give rise to undesirable symptoms in the animals at the levels at which they must be fed to supply minimal amounts of the vitamin. It is true that maize might be balanced by a few other foodstuffs on the market, but this tends to restrict the farmer in his choice of the cheaper foods. Nor should alfalfa meal exceed 6 per cent. of the ration, for at this level it exhibits a tendency to lower the net energy value and even 6 per cent. of certain alfalfa meals on the market may be insufficient as a source of vitamin A for the rapidly growing animals.

The simplest and most foolproof solution to the problem would appear to be the dosage of the young animals with a potent concentrate. One large dose of 1,000,000 I.U.—or two doses of 500,000 I.U., one at time of castration (6 weeks) and the second at weaning (8 weeks)—will be sufficient to ensure normal growth to bacon weights with an appreciable reserve still in the liver at slaughter, irrespective of the type of ration used. Such a practice would undoubtedly lead to a great decrease in the mortality and unthriftiness of swine and also enable the farmer to dispense with the daily or weekly additions of expensive and unstandardised cod-liver oil to the diet of his animals. A method similar to this was adopted with marked success in a previous experiment (5). Animals so treated grew normally and still retained over 100 B.U. per gm. in their liver at bacon weight despite the fact that the diet was grossly deficient in vitamin A, as was indicated by the state of collapse in the untreated animals.

From our results it would appear that a ration containing 15 per cent. of the maize meal and 3 per cent. of the alfalfa meal we used would be sufficient to prevent depletion of the animals' reserves and at the same time to guard against the undesirable properties of these foodstuffs when they are much in excess of these amounts. As we have indicated, however, the inclusion of these amounts cannot be recommended to meet the vitamin A requirements of swine till both foods are standardised or are marketed along with the results of an official analysis of their carotene contents.

THE VITAMIN A REQUIREMENT.

The carotene content of the rations has been calculated from the data in Table I. The fish meal used must have contained appreciable amounts of vitamin A, but these were not determined. All the other deficient diets, however, contained less than 40 mg. of carotene per 100 lb. of the ration, while Nos. 7, 8 and 10 contained 56·8, 89·2 and 60·2 mg. respectively. If we take the latter diet as representing the minimal requirements of swine for vitamin A, then, adopting Sherman's practice of allowing a factor of safety—50 per cent., all rations should contain at least 90·3 mg. carotene per 100 lb. to keep the vitamin A reserves of the animal at their original level.

In other words, a 100 lb. pig should have the equivalent of 4 mg. carotene in its diet to satisfy its vitamin A requirement for maintenance and normal growth (1·3 lb. per day).

With regard to the chemical constitution of the carotene it is only possible to say that it is the carotene from mixed meals. This is unlikely to be pure α, β or γ carotene, being probably a mixture of these. Evidence is accumulating, however, that β carotene is the commonest form present in foodstuffs (10).

The vitamin solution used contained 600,000 I.U. in 1 litre or 600 I.U. per ml. Five ml. were added to the diet of the deficient animals for therapeutic purposes, so that each received 3000 I.U. vitamin A (equivalent to 2 mg. of carotene) from this source per day. The effect of this was to increase the vitamin A potency of the deficient diets from under 40 mg. carotene to between 70 and 85 mg. per 100 lb. As will be seen from the analyses of the livers of the animals at slaughter, these supplemented diets were only little more than sufficient for recovery. The storage is extremely small and may be contrasted with the results of Golding and Foot (9) who obtained storage of 1000 I.U. per gm. of liver after recovery. In their case, however, the therapeutic doses were excessive in so far as the minimum necessary requirements are concerned. The disease symptoms in their animals disappeared within 1 week, some recovering in 3–4 days, and further dosing only served to increase the liver reserves.

SUMMARY AND CONCLUSIONS.

The present paper reports the results of experiments which were carried out to obtain information on the types and amounts of the common foodstuffs which should be included in a ration for the prevention of avitaminosis A in swine. The actual vitamin A requirement of swine in terms of carotene was also investigated.

Ten different diets were used and three animals were placed on each. Seven of these diets produced the classical symptoms of avitaminosis A in the animals, two contained minimal or just subminimal amounts of the vitamin, while one appeared to permit storage.

The rations on which the symptoms of avitaminosis A did not appear included maize meal and alfalfa meal. Both these foodstuffs, however, have been shown to produce undesirable effects in the animals at the levels at which they must be fed to prevent the onset of the disease symptoms.

Neither of them are standard products—the vitamin A content of the maize varying with the number of genes the original crop carried for the yellow factor and with time and methods of storage, while the potency of alfalfa meal is known to be dependent on the method of curing which in this country destroys most of the vitamin.

Since neither of these foods can be recommended with confidence, the dosing of the young animals with a concentrate of known potency (1,000,000 I.U. vitamin A per animal) appears to be the simplest and most foolproof method for prevention of avitaminosis A under intensive conditions of management. The fattening ration may then be composed of the cheapest foodstuffs irrespective of their vitamin A content.

On a diet high in maize growth ceased in all three animals. Small supplements of yeast proved sufficient for recovery and resumption of normal growth. It is suggested that the failure of the animals to grow was due to a deficiency of vitamin B_2 in their diet.

Four animals died during the experiment and post-mortem examination showed extensive enteritis. All these animals were shown to have depleted their body reserve of vitamin A.

The amount of vitamin A necessary in the diet to keep the animals' reserves at their original level has been shown to be approximately 60 mg. carotene per 100 lb. of food.

Or, alternatively, the vitamin A requirement of a 100 lb. pig for maintenance and normal growth (1·3 lb. per day) is 4 mg. of carotene per day.

The writer desires to thank Dr Alan W. Davies who kindly undertook the analysis of certain samples of liver for vitamin A during an enforced absence of the writer. He is indebted to Mr A. L. Bacharach both for generous gifts of the vitamin concentrate and for kindly offering to carry out duplicate examinations of the liver samples at the Glaxo Research Laboratories in London. His thanks are also due to Mr E. A. Porter for the care and attention he devoted to the animals.

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(*Received 4th January*, 1935.)

INTERVARIETAL COMPETITION IN YIELD TRIALS WITH COTTON.

By BASIL G. CHRISTIDIS.

(Of the Greek Cotton Institute, Salonica, Greece.)

(With One Text-figure.)

THE IMPORTANCE OF COMPETITION.

THE effect which different varieties grown on small plots side by side may have upon each other is usually termed competition. Naturally if a tall variety, or one having a root system highly developed, is planted on small plots in alternation with a short variety or one with small roots, it is to be expected that the latter will suffer from the unfavourable neighbourhood, whereas the former will be benefited. In other words, when testing varieties differing in their habits of growth, etc., owing to the effect of competition some of them may appear better and others worse than they are under natural conditions.

This disturbing factor has been studied by several authors (Montgomery (8), Hayes and Arny (3), Kiesselbach (5), Love (7), Stadler (9), Stringfield (10)). The results of their work, which have been reviewed by the present writer elsewhere ((11), pp. 30–1), although greatly diverging, seem to point to the general conclusions that: (1) competition does exist, at least in some cases, (2) its effect (if any) is limited to only one row on each side of the plot, and (3) when varieties similar in their habits of growth are grouped together, the effect of competition may be negligible.

In a recent paper by Immer (4) it was shown that so far as yield per acre is concerned, the effects of competition were apparent when two varieties of sugar-beet differing in size of plants were grown on alternative single-row plots. These results were obtained by comparisons made in two separate trials, which were placed adjacent to each other in the same field. Ligon (6) showed that in field experiments with cotton a three-row plot in which the central row only is used for yield data might serve as well as plots with more rows. Coombs (1) studied the importance or otherwise of rejecting edge rows in experiments on kale and sugar-beet having small or narrow plots. He found that the retention of the edge rows would give decidedly biased estimates of the treatment differences; on the other hand he came to the conclusion that if the treatment is a manurial one the discarding of edge rows might give more biased results than their retention, owing to the unequal distribution of the manures between the centres and outsides of the plots.

From a practical point of view it is of considerable importance whether three-row or single-row plots should be adopted. In the case of three-row plots two-thirds of the whole experimental area available are wasted. Moreover, the rows to be compared are not adjacent, being separated by two border rows, which presumably causes a decrease in the correlation between neighbouring plots.

PURPOSE AND LAY-OUT OF THE PRESENT EXPERIMENTAL WORK.

In order to test whether single-row plots could be employed by the Greek Cotton Institute in its experiments with various cotton varieties,

Table I.

No. of plot	Variety sown	Yield of seed cotton in kg.				Mean height of plants in cm.			
		Rows				Rows			
		a	*b*	*c*	Whole plot	*a*	*b*	*c*	Whole plot
1	Se.	14·94	16·59	17·32	48·85	62	65	68	65
2	A.	13·95	13·75	13·54	41·24	63	62	59	61
3	Su.	11·40	11·16	11·85	34·41	55	54	52	54
4	D.	13·97	14·34	13·50	41·81	54	49	54	52
5	T.	12·62	17·26	13·74	43·62	55	54	58	56
6	Cl.	10·96	9·66	9·58	30·20	55	57	55	56
7	Se.	14·13	14·50	14·31	42·94	56	57	58	57
8	Cl.	13·17	13·34	12·41	38·92	56	57	56	56
9	I.	15·74	10·41	13·13	39·28	63	62	61	62
10	C.F.	9·46	14·11	13·11	36·68	62	63	62	62
11	Su.	11·06	11·90	11·79	34·75	58	50	43	50
12	I.	11·66	11·81	12·77	36·24	50	52	52	52
13	C.F.	13·26	14·34	13·41	41·01	56	56	52	55
14	A.	12·88	12·26	12·52	37·66	51	50	51	51
15	K.	12·55	12·23	12·52	37·30	51	58	52	54
16	T.	12·70	13·25	13·68	39·63	45	49	48	47
17	K.	14·02	9·66	12·53	36·21	52	52	51	52
18	D.	14·97	11·83	11·69	38·49	47	47	43	46
19	Su.	12·23	12·56	12·81	37·60	47	46	42	45
20	C.F.	12·94	13·83	12·02	38·79	49	56	54	53
21	I.	9·53	9·62	9·84	28·99	42	46	48	45
22	A.	9·60	9·24	9·03	27·87	47	48	43	46
23	Se.	10·10	10·03	10·86	30·99	44	50	45	46
24	C.F.	9·62	9·59	9·21	28·42	47	44	45	45
25	Su.	9·71	10·22	9·75	29·68	43	42	42	42
26	D.	9·58	9·42	9·05	28·05	49	42	51	47
27	I.	9·45	9·39	9·83	28·67	49	51	49	50
28	Cl.	9·91	9·15	9·64	28·70	46	47	51	48
29	A.	9·73	9·81	9·93	29·47	50	48	45	48
30	T.	11·59	12·06	11·86	35·51	51	46	55	51
31	Cl.	8·70	8·84	8·65	26·19	51	54	54	53
32	Se.	15·81	10·05	13·53	39·39	55	49	47	50
33	D.	12·26	11·75	10·98	34·99	45	49	53	49
34	K.	13·90	13·19	11·97	39·06	51	52	51	51
35	K.	12·57	12·07	12·48	37·12	51	50	46	49
36	T.	13·27	12·65	11·26	37·18	48	47	46	47
37	D.	13·30	13·98	13·04	40·32	50	48	51	50

Table I (*cont.*)

No. of plot	Variety sown	Yield of seed cotton in kg.				Mean height of plants in cm.			
		Rows			Whole plot	Rows			Whole plot
		a	*b*	*c*		*a*	*b*	*c*	
38	Cl.	13·18	12·70	13·51	39·39	56	52	56	55
39	T.	13·56	14·28	12·65	40·49	56	49	48	51
40	Su.	12·21	11·98	12·96	37·15	49	55	53	52
41	K.	12·92	13·75	13·08	39·75	58	59	56	57
42	I.	13·67	14·31	13·64	41·62	61	57	57	58
43	Cl.	12·73	16·21	14·67	43·61	66	61	55	61
44	T.	17·14	15·75	14·64	47·53	56	59	60	58
45	Se.	15·17	15·12	14·82	45·11	51	58	57	55
46	Se.	14·57	13·34	14·92	42·83	57	56	55	56
47	Su.	12·31	12·93	11·78	37·02	51	60	55	55
48	A.	12·75	11·76	10·56	35·07	59	51	52	54
49	A.	11·78	12·92	10·92	35·62	57	57	58	57
50	I.	11·85	12·28	12·59	36·72	55	49	53	52
51	C.F.	10·07	11·72	10·81	32·60	50	48	50	49
52	C.F.	10·62	10·45	9·52	30·59	51	52	50	51
53	K.	9·77	10·44	10·85	31·06	54	45	43	47
54	D.	11·30	11·46	10·87	33·63	45	46	43	45
55	T.	9·71	11·06	11·71	32·48	46	46	46	46
56	K.	10·44	10·36	11·10	31·90	49	48	46	48
57	Cl.	8·24	7·50	6·99	22·73	53	43	43	46
58	D.	9·72	10·69	10·70	31·11	45	48	50	48
59	C.F.	9·78	10·54	10·98	31·30	51	51	50	51
60	I.	10·96	10·44	10·68	32·08	48	47	46	47
61	T.	8·86	10·69	10·49	30·04	47	48	50	48
62	C.F.	11·84	10·54	9·89	32·27	49	51	51	50
63	Se.	10·53	9·73	10·08	30·34	49	52	47	49
64	A.	9·30	8·85	9·83	27·98	49	45	44	46
65	Su.	10·85	11·18	10·16	32·19	46	46	50	47
66	A.	9·23	9·03	8·44	26·70	46	48	47	47
67	Se.	9·66	9·23	10·11	29·00	48	45	44	46
68	D.	9·04	9·49	10·54	29·07	50	48	46	48
69	K.	10·48	9·68	9·02	29·18	43	44	46	44
70	I.	9·06	8·96	9·17	27·19	47	53	52	51
71	Cl.	8·37	8·33	9·32	26·02	50	43	43	45
72	Su.	12·33	10·84	10·60	33·77	43	41	43	42
73	Su.	9·62	9·42	9·37	28·41	39	43	42	41
74	T.	9·70	10·14	8·67	28·51	43	43	42	43
75	C.F.	8·78	8·97	8·57	26·32	41	41	39	40
76	Su.	9·75	9·06	9·09	27·90	39	42	42	41
77	K.	9·08	9·20	8·81	27·09	46	45	44	45
78	Cl.	8·83	9·13	9·11	27·07	45	41	46	44
79	Cl.	8·56	8·23	7·76	24·55	46	44	47	46
80	Se.	10·35	10·11	10·27	30·73	42	41	44	42
81	A.	8·68	8·27	8·18	25·13	41	39	43	41
82	D.	8·85	9·02	9·36	27·23	38	38	39	38
83	T.	10·11	9·48	9·71	29·30	40	42	40	41
84	D.	9·97	10·44	8·76	29·17	40	37	43	40
85	K.	9·77	8·92	8·90	27·59	46	41	41	43
86	A.	9·31	9·29	8·38	26·98	31	45	45	40
87	I.	9·27	9·59	8·88	27·74	41	44	45	43
88	Se.	9·92	9·91	10·43	30·26	45	47	43	45
89	C.F.	9·71	9·89	10·56	30·16	48	47	52	49
90	I.	10·66	10·34	10·54	31·54	46	51	51	49

I. = Ingold.
T. = Trice.
K. = King.
D. = Delfos.
A. = Acala.
Cl. = Cleveland.
C.F. = Carolina Foster.
Su. = Sunshine.
Se. = Serres (local).

the following experiment has been carried out at the Verria Cotton Sub-Station in 1932.

Nine varieties, which preliminary trials showed to be of varying economic importance for the cotton grower, were used in an ordinary variety test. The plots were 100 m. long and 2·40 m. wide, each having three rows 0·80 m. apart. The distances between plants within the row were 0·30 m., and after thinning two plants were left per hill. The experiment was planned according to the randomised block system, with five blocks. In each block, however, every variety was planted at random twice (with the original intention of trying two different treatments of seed), so that there were ten replications of the nine cotton varieties mentioned in Table I.

At harvest every row was picked and weighed separately, and after the leaves of the plants had dried up and fallen the height of the main stem of every plant was measured also. The results obtained are shown in Table I.

Testing the effect of competition.

(*a*) *Yield data.*

On general grounds competition may be expected to affect the comparative yielding values of rows *a*, *b* and *c* (see Fig. 1), in the following manner:

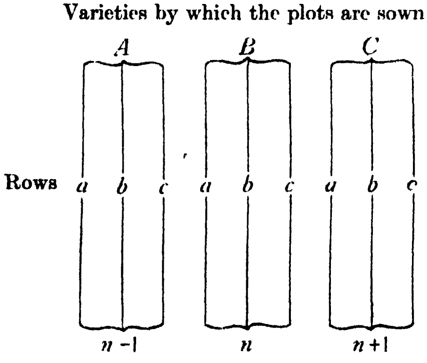

Figure 1. Notation for plots, rows and varieties.

(1) If varieties A and C are both better competitors than variety B, the yield of the middle row b of plot n will be greater than the yields of its border rows a or c.

(2) If varieties A and C are both poorer competitors than variety B, the middle row b of plot n will yield less than its respective border rows a or c.

(3) Finally, in the case of variety A being better and variety C being worse than variety B, or *vice versa*, the yielding value of row b of plot n, should be intermediate to the yields of rows a and c.

In other words, in case (1) the differences in yield of rows a–b and c–b would be both positive, and the magnitude of their mean in proportion to the competitive superiority (with regard to yield) of variety B compared with A and C. In case (2) these differences would be both negative, and the magnitude of the mean would show the inferiority of variety B in comparison with A and C. Finally, in case (3) the mean of differences a–b and c–b would tend to be small, unless variety A is much stronger in comparison to variety B, and variety C only slightly weaker, or *vice versa*. If there are different competitive values between the cotton varieties under investigation, the means of the differences $\frac{1}{2}(a+c-2b)$ will measure the competitive strength of the various varieties.

Accordingly the means of the differences $\frac{1}{2}(a+c-2b)$ were worked out from the data of Table I and classified according to variety. The analysis of the differences $\frac{1}{2}(a+c-2b)$ is given below (block differences are not eliminated).

	D.F.	S.S.	Variance	S.D.	$\log_e$ S.D.
Varieties	8	19·8871	2·4859	1·5767	0·45540
Error	81	89·2550	1·1019	1·0497	0·04850
Total	89	108·8060			

$z=0{\cdot}40690$ which is significant, since for $n_1=8$, $n_2=81$ and $P=5$ per cent., $z=0{\cdot}3356$.

The S.D. of a difference between two means of ten observations each being in this case $\sqrt{\frac{2\times 1{\cdot}1019}{10}}$ or $\pm 0{\cdot}469$, statistically significant differences would be those exceeding $\pm 0{\cdot}919$ $(=1{\cdot}959\times 0{\cdot}469)$. The mean differences $\frac{1}{2}(a+c-2b)$ for each variety, as well as the average yield per plot, is given in Table II, from which it may clearly be seen that several of the mean differences are significantly different.

Varieties Serres, Ingold and King when grown in competition may exceed in yield varieties Carolina Foster and Trice by 9–12 per cent. simply owing to their better competitive value. This percentage would presumably be much larger (particularly regarding the extreme competitors Serres and Trice), if single-row plots were used, when competition is taking place on both sides of each row.

(*b*) *Height data.*

The foregoing considerations apply also to the heights of the plants. The differences $\frac{1}{2}(a+c-2b)$ have been determined per plot and their

Table II. *Average yield per plot in kilograms and mean differences* $\frac{1}{2}(a+c-2b)$.

	Yield				Differences $\frac{1}{2}(a+c-2b)$	
Variety	Whole plots	Row *a*	Row *b*	Row *c*	Mean	% of row *b*
Se.	37·04	12·52	11·86	12·67	0·731	6·13
I.	33·01	11·19	10·72	11·11	0·431	4·02
K.	33·63	11·55	10·95	11·13	0·388	3·54
Su.	33·29	11·15	11·13	11·02	−0·044	−0·40
A.	31·37	10·72	10·52	10·13	−0·091	−0·87
Cl.	30·74	10·27	10·31	10·16	−0·095	−0·92
D.	33·39	11·30	11·24	10·85	−0·170	−1·51
C.F.	32·81	10·61	11·40	10·81	−0·690	−6·05
T.	36·43	11·93	12·66	11·84	−0·779	−6·15
General mean	33·52	11·25	11·20	11·08	−0·035	−0·31

averages for each variety, as well as the mean heights of plants, are shown in Table III. Their statistical analysis is as follows:

	D.F.	S.S.	Variance	S.D.	$\log_e$ S.D.
Varieties	8	90·9972	11·3747	3·3925	1·22158
Error	81	623·4917	76·9743	8·7735	2·17173
Total	89	714·4889			

z = not significant.

In this instance competition is not apparent. This may be explained by the fact that competition effects are counteracted by a stimulation to growth in height owing to shading.

Table III. *Average height of plants per plots in cm. and mean differences* $\frac{1}{2}(a+c-2b)$.

	Height of plants				Differences $\frac{1}{2}(a+c-2b)$	
Variety	Whole plots	Row *a*	Row *b*	Row *c*	Mean	% of row *b*
D.	46·3	46·3	45·2	47·3	1·60	3·53
Cl.	51·0	52·4	49·9	50·6	1·60	3·21
T.	48·8	48·7	48·3	49·3	0·70	1·45
A.	49·1	49·4	49·3	48·7	−0·25	−0·51
I.	50·9	50·2	51·2	51·4	−0·40	−0·78
C.F.	50·6	50·4	50·9	50·5	−0·45	−0·88
K.	49·0	50·1	49·4	47·6	−0·55	−1·11
Se.	51·2	50·9	52·0	50·8	−1·15	−2·21
Su.	47·1	47·0	47·9	46·4	−1·20	−2·51
General mean	49·34	49·49	49·34	49·18	−0·01	−0·02

CONCLUSIONS.

1. Competitive effects have been detected in a field test with nine varieties of cotton.

2. The results obtained by the yield data showed a variation ascribed to competition, which varied from zero to ± 6 per cent. of the mean (in rough figures). Two groups can definitely be distinguished as including

the best and the worst competitors. However, the best yielder is not always the best competitor or *vice versa.*

3. The height-of-plants data do not show any indication of competition, presumably owing to an opposite effect of shading.

4. The competitive value of a variety, as estimated in the preceding paragraphs, depends on that of the other varieties, with which it is grown in competition.

5. The results of the above experiment seem to suggest that competition may cause a definite bias in estimating the comparative yielding value of cotton varieties. Therefore it appears advisable that field trials should be so arranged that competition effects between different varieties will be eliminated.

Many thanks are due to Professor R. A. Fisher, of University College, London, and Mr F. Yates, of Rothamsted Experimental Station, who gave considerable help and guidance in connection with the statistical analysis of the experimental data.

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(*Received January 8th,* 1935.)

AN EXAMINATION OF THE VALUE OF COVARIANCE IN DAIRY COW NUTRITION EXPERIMENTS.

By M. S. BARTLETT.

(*Statistician, Imperial Chemical Industries, Agricultural Research Station, Jealott's Hill, Bracknell, Berks.*)

I. Introduction.

The use of covariance as a statistical method of adjusting experimental observations by means of previous data is by now fairly well known. Its value promises to be greatest where the experimental units exhibit most individuality, correction for which will reduce the variation contributing to error and increase the efficiency of the experiment. Just as the method of "randomised blocks" in field experiments, for eliminating differences in soil fertility while preserving a valid estimate of error, associates the statistical analysis of an experiment with its actual design, so it is inevitable that as the value of covariance becomes more widely appreciated, it will when advantageous be deliberately incorporated in the experimental technique. Thus Murray (1) has suggested making a year's uniformity trial with rubber trees before any application of treatments; and Yates (2), by recording initial pig live weights, has been able to ignore these weights until the final analysis when grading pigs into "blocks" for a feeding trial.

The individuality of the milk yield of a dairy cow seems to present a somewhat complex problem; it exists not only in the average or total amount of milk produced, but also in the shape of the lactation curve. This has caused experimenters in work on nutrition to try out methods different from those that would normally be employed, and adopt a change-over system of treatments rather than maintain one cow or group of cows entirely on one treatment. Statistically valid experiments of this kind can be designed, and are capable of giving very accurate comparisons. Certain obvious criticisms of this type of experiment must, however, be made. Apart from the drawback that the number of treatments would ordinarily have to be limited to two, it will be noticed that the treatments can only be tested for comparatively short periods, and that it is in fact only this limitation on length of period per treatment that has ensured accuracy in the treatment comparisons. The objection can always be made that the experiment does not correspond to practice.

Such considerations make it worth while seeing how far an experiment might be designed on normal lines, and yet have sufficient efficiency to be of use. For this purpose data are analysed here which really correspond to an experiment of the change-over type, being the results of an experiment carried out at Jealott's Hill on the nutritive value of dried grass for winter feeding as compared with the ordinary ration. Since, in this experiment, which was extremely accurate, no significant difference in milk yield was observed, its results are of value, apart from their original purpose, when the experiment is regarded purely as a uniformity trial. The experimental period of twenty weeks was actually divided into four subperiods of five weeks each; but it is sufficient here to note that the records of the milk yields are available per week for each of the twenty cows in the experiment. These cows consisted of four breeds: eight Shorthorns, four Ayrshires, four Friesians and four Guernseys. Their feeding was rationed, consisting of a maintenance ration based on live weight and a production ration proportional to the milk yield. All the cows had calved not more than about twelve weeks previous to the beginning of the experimental period. For further particulars reference must be made to the original description of this experiment (3).

II. ANALYSIS OF DATA AVAILABLE, WITH REFERENCE TO THE USE OF COVARIANCE.

The data in the form required for this investigation are given in Table I. The quantity x_n denotes the average milk yield in lb. per week

Table I.

Breed	Cow	y_0	x_1	y_1	x_2	y_2	x_3	y_3	x_5	y_5	y_{15}
Shorthorn	Betty	191	233	189	226	187	223	185	218	182	154
	Hollyhock	318	329	317	320	318	324	317	328	314	290
	Queenie	175	206	173	201	172	200	171	191	169	160
	Kathy III	236	296	233	294	230	294	226	289	219	204
	Olive	180	204	179	204	178	204	176	197	175	164
	Martha	196	251	193	246	190	238	188	228	185	172
	Cherry	166	187	165	190	163	192	161	188	158	149
	Gladys	212	243	210	237	209	237	208	238	203	180
Friesian	Pearl	238	289	235	286	233	284	230	279	224	195
	Saucy	196	241	193	235	191	230	189	224	186	172
	Dairymaid	281	353	278	344	276	339	271	332	265	253
	Fancy	260	307	257	302	255	293	254	284	252	222
Ayrshire	Ninna	364	414	361	412	359	413	355	412	348	300
	Tiny	187	207	186	205	185	204	184	200	182	172
	Grace	191	211	190	213	189	212	187	206	186	162
	Ruth	161	208	159	207	156	204	154	197	149	131
Guernsey	Nellie	187	233	184	226	183	221	181	215	177	171
	Bessie	177	258	172	253	168	244	165	230	159	132
	Eva	194	234	191	232	189	228	188	220	185	168
	Lottie	192	217	191	224	189	225	187	223	182	180

for the first n weeks of the experimental period, y_n the average milk yield for the remaining 20—n weeks.

A straightforward analysis of variance of y_0 gives the following result.

Table II.

Variation	D.F.	Sums of squares	Variance
Between breeds	3	7,058	2353
Within breeds	16	47,110	2944
Total	19	54,168	—

After eliminating differences between breeds, the standard error per cow is estimated to be 54·26, or 25·23 per cent. of the general mean. Actually, of course, if an experiment had been conducted on a "randomised block" basis with only two treatments, the breeds would have been subdivided into groups of two, and some of the variation within breeds eliminated as block differences. This would not, however, have reduced the standard error to a level worth considering, especially as the cows included one or two anomalous high-yielders.

An analysis of variance and covariance was next made on the mean yields x_5 and y_5.

Table III.

	D.F.	x_5^2	x_5y_5	y_5^2
Shorthorn	7	17,499·9	16,337·9	17,261·9
Friesian	3	5,856·8	4,379·8	3,668·8
Ayrshire	3	33,432·7	27,951·2	23,968·7
Guernsey	3	118·0	− 155·0	406·7
Between breeds	3	8,113·6	7,166·1	6,943·9
Total	19	65,021·0	55,680·0	52,250·0

From this table may be calculated the regression coefficient of y on x for each breed separately. The corrected variance within breeds is shown in Table IV with a separate adjustment for each breed and, since the differences between the regression coefficients are not significant, is shown also when adjusted by means of the common regression coefficient for all the cows, apart from variation in the breed means. The sum of squares appropriate for testing the differences between breeds is obtained by the recognised procedure (see (4), p. 267, or (5), p. 333), being the sum of

Table IV.

	D.F.	Sums of squares	Variance
Within breeds (separate regression coefficients)	12	3205·7	267·1
Between breed regression coefficients	3	742·0	247·3
Within breeds	15	3947·7	263·2
Between breeds	3	621·3	207·1
Total	18	4569·0	—

squares for the variation between breeds corrected by means of the regression coefficient obtained from the means, plus the square representing the difference between this coefficient and the common coefficient obtained from the variation within breeds.

Taking the variance 263·2, with the corresponding standard error 16·22, we see that the error has been very much reduced. It is now 7·91 per cent. of the general mean of the last fifteen weeks and, although this error is not so low as one might wish, it is at least of the same order as errors in field experiments. The efficiency of a straightforward continuous treatment experiment would with this material have been increased five or tenfold if a control period of five weeks had been allowed before the treatments proper began.

How far it is necessary to allow as much as five weeks as a preliminary period was investigated by examining the standard error obtained if one, two or three weeks only had been given. Further, since in an actual experiment if treatments were causing any cumulative effect, this effect might be most marked at the later stage of the experimental period, the standard error of the last five weeks when adjusted for the first five was also calculated. Table V summarises the results, the standard errors being expressed as percentages of the general mean of the period considered corresponding to experimental treatments, and obtained in a similar manner to the figure 7·91 computed above.

Table V. *Percentage standard errors.*

				Five weeks' control	
No adjustment	One week's control	Two weeks' control	Three weeks' control	Fifteen weeks' exp.	Last five weeks
25·23	8·22	8·53	8·14	7·91	9·88

The percentage standard error for one week's control happens to be less than for two weeks. Not much is gained by extending the control period to more than one week; it would seem inadvisable, however, to reduce it too far, owing to possible temporary fluctuations in milk yield, and three weeks appears to be a satisfactory period.

III. DISCUSSION.

The analyses above have been made with a view to seeing how far an efficient experiment can be designed without recourse to any changeover of treatments, apart from the inevitable change from ordinary ration to a particular treatment at some stage at the beginning of the experiment. This initial change must, of course, always be made as gently as possible, to avoid apparent effects of treatment which may be merely effects of change in ration. Inferences on the efficiency of future experiments conducted on lines indicated by the results here will only be true in so far as the material investigated is in its variation representative of nutrition experiments on dairy cows in general. Most confidence can therefore be expressed in the applicability of the results to winter nutrition experiments with rationed feeding of the same kind as the original experiment from which the data were obtained. Although the cows varied considerably in their level of milk yield, an attempt was made in this experiment to obtain cows at a stage not only when a twenty weeks' experimental period was possible, but also just after the usual initial rise in milk yield at the beginning of lactation was over, and a steady drop per week could be assumed.

Let us suppose that an experiment is decided on with three weeks' control ration followed by as many weeks' treatment ration as is practicable, cows being available which have calved only a few weeks previously. It should be noted that even if no control period had been allowed for, presumably a record of the milk yield for the week previous to the experimental period would be available, and could be used instead. The rôle of covariance as a factor in the design of the experiment implies, however, that the control period should be as much a part of the experiment as the experimental period proper; this will, for example, ensure that any disturbances such as rearranging the animals in the shed or keeping them entirely confined indoors occur before the initial yield is recorded as well as before the various treatment yields are obtained.

The cows would be selected into groups with the number per group corresponding to the number of treatments. Provided that the cows within each group do not differ violently in level of milk yield, we may anticipate that, after adjustment for initial yield, the chief residual variation will be due to differences in the general slope and character of the lactation curve. If possible, therefore, selection into groups should be made with a view to keeping together cows likely to have similar lactation curves. Thus it would be natural to separate heifers from aged cows, and cows already calved for, say, ten weeks, apart from cows freshly calved and still showing a slight increase in their milk yields. It might even be advisable, if a group of freshly calved cows showed different increases in yield, to delay the experiment proper with this group until a reasonably uniform control period was reached.

In this discussion it has been assumed that nothing may be known about the cows apart from the usual facts such as existing level of milk yield and approximate date of calving. This was in fact the case with most of the cows whose yields have been considered above, these cows being mostly especially bought in for the experiment. Previous records of complete lactations, if available, may prove of value as well as or even instead of initial yields; it has not been possible to investigate this point here. But the necessity of having a sufficient number of cows calving at the right period for a winter experiment of sufficient duration to be possible will mean, in many cases, that such information for all the cows will not be available, some of the cows being bought in. Where such information does exist, if only for some of the cows, it can, of course, always be used when the cows are being graded into groups.

The procedure of allotting the treatments at random to the cows of each group, and conducting the experiment on statistically valid lines need not be gone into here; those unversed in these requirements may refer, for example, to (2).

Some consideration is needed on the most efficient analysis of experiments conducted on the above lines, and it is not possible to decide finally from the limited data examined here what is to be the exact form that the analysis should take. In the calculation above of the standard error per cow the differences between regression coefficients per breed were insignificant and ignored. It is probable, though groups of cows may have been selected so that the character of lactation curves may differ somewhat between groups, that the elimination of block means together with the common regression coefficient will be sufficient, possible differences between blocks being mainly eliminated by taking out block means. The logical alternative appears to be to treat the problem entirely as one of partial regression, calculating regression coefficients rather than means both for blocks and treatments. The analysis would then, however, become more complex, the orthogonality of the design for simple means being given up. Further, it is desirable that the results should be readily interpretable, and differences in mean yields convey more to the layman than statements about regression coefficients. With large samples and small treatment differences, the two methods of analysis must, from general considerations, be approximately equally efficient, but with limited data from which we wish to establish the reality of possibly fair-sized treatment differences, they cannot be assumed altogether equivalent.

In conclusion, it must again be stressed that the suggestions put forward here must be regarded as provisional, both with regard to the design of the experiment and to the analysis of the results, until any value they may have has been demonstrated by actual experiment. The data examined are limited, and are not entirely in the form required for a uniformity trial. With regard to the analysis, it cannot be foreseen how far the standard error calculated from experimental data with actual treatment differences may, on the usual analysis of corrected means, be larger than necessary.

An actual experiment of this type was begun at Jealott's Hill at the end of 1934, and its results, when available, will furnish more direct evidence of what accuracy we may expect to attain.

IV. SUMMARY.

The results of a winter nutrition experiment on dairy cows were used as a uniformity trial in order to indicate the magnitude of the standard error we may expect if a continuous treatment experiment is designed with a preliminary control period.

An initial period of three weeks is suggested, and some discussion given on the design and analysis of this type of experiment.

V. ACKNOWLEDGMENTS.

I am indebted to Dr S. J. Watson, who supplied and discussed with me the material considered in this paper; and to Mr F. Yates, with whom I also was able to discuss the data while staying at Rothamsted.

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[1] This report is available on loan, on application to Jealott's Hill.

(*Received January 9th*, 1935.)

PRELIMINARY EXPERIMENTS ON VERNALISATION.

By G. D. H. BELL, Ph.D.

(*Plant Breeding Institute, School of Agriculture, Cambridge.*)

(With Four Text-figures.)

Introduction.

The experiments described in this paper were primarily designed to test the reactions of some cereal varieties to Lyssenko's vernalisation seed pre-treatment. No attempt has been made to investigate critically the physiological problems involved, because it was not known whether any of the varieties used would respond to the treatment under the growing conditions of this country. It is therefore desired to emphasise that the results obtained are of a preliminary nature. The tables included in the paper are not intended to lend statistical accuracy to the results, but serve merely to make the text description more intelligible.

The term "vernalisation" was coined by Whyte and Hudson (1) to describe the method of seed pre-treatment devised by Lyssenko at the Odessa Plant Breeding Institute in 1932. Briefly stated, Lyssenko's work shows that by appropriate treatment of the seed in its first stages of germination, the subsequent vegetative phase of the plant can be shortened. This means that the plant is stimulated to flower earlier. Thus, by exposing the germinating grain of spring wheat to temperatures between 3 and 5° C. for 10 days, Lyssenko succeeded in bringing the wheat into ear 5–10 days earlier than untreated samples of the same variety.

It is claimed that the stimulus for early earing in the case of wheat, and other long-day plants, is the low temperature. By sowing early in the spring, or in the winter, the wheat is exposed to the necessary low temperature. Later spring sowings are not subjected to this low temperature exposure, and earing is consequently greatly retarded, or may even fail entirely, as in the case of late spring sown winter-wheat. In order to retain the benefits of early sowing, where such a practice is impracticable or impossible, the grain is exposed to low temperature before sowing by artificial treatment, or "vernalisation".

The physiological significance of this is that the plant is apparently capable of physiological development or change without morphological growth. Demkovsky (1) has shown that vernalised seeds show an increased enzyme activity: while Lyssenko found that a vernalised plant develops more dry matter per unit of time than an untreated plant. Low temperature thus appears to be capable of stimulating the plant metabolically, and this stimulation, though applied to the plant in the embryonic stage, affects the subsequent development.

MATERIAL AND METHODS USED IN THE PRESENT EXPERIMENTS.

The following cereal varieties were used:

Barley (winter forms): Wintergerste, Bocumer, *H. praecox*, *H. spontaneum.*

Barley (spring forms): Goldthorpe, Big-wheat Barley, Swanneck, Pryor's Chevallier, Spratt-Archer, *H. haxtonii.*

Wheat (winter forms): Cambridge Rivett, Joss IV, Yeoman II.

Wheat (spring forms): April Bearded.

Oats (winter forms): Grey Winter.

Oats (spring forms): Abundance, Argentine, Bountiful, Marvellous.

Small samples of about 300 grain of each variety were placed in germinating dishes and approximately 50 per cent. by weight of distilled water added to each. After stirring, to ensure that each grain was moist, the dishes were covered and left at room temperature (about 60° F.) for 24 hours. After this preliminary treatment the dishes were transferred to dark storage chambers at the Low Temperature Research Station. Lyssenko claims that no more water need be added after the initial 50 per cent., but in the present experiments a few drops were added every other day to prevent drying out of the germinating embryos. The bulk of the grain was kept at 3° C., but some varieties were also stored at 1° C. Three periods of exposure were used, viz. 7, 14 and 21 days.

SOWING.

The abnormally dry winter of 1933–4, followed by an equally dry early spring, made early sowing desirable before the ground became too dry for sowing the partially germinated grain. Accordingly, it was decided to sow on March 1st, which is 2–3 weeks earlier than is desirable for this experiment. Because of this early sowing, half the grain was kept in the low temperature chambers for one more week to allow of another sowing on March 8th. In addition to these two early sowings, an abnormally late sowing was made on April 28th, with grain treated for 14 days at 3° C.

In addition to these field sowings, some of the varieties from the March 8th sowing were sown in pots in an unheated glasshouse. From March 8th to April 15th the pots were kept in the glasshouse for the full 24 hours each day. From April 16th to 25th the pots were placed outside the glasshouse during the day, and brought in at night. From April 26th the plants were allowed to remain outside for the whole of the 24 hours each day. The object of this treatment was to avoid exposing the young plants to any low temperature, even of the order experienced in early spring in this country.

The following treatments and sowing times have to be considered:

March 1st sowing (field):

(1) Grain kept for 14 days at 3° C.
(2) ,, 7 ,, 3° C.
(3) ,, 14 ,, 1° C.

March 8th sowing (field and glasshouse):

(1) Grain kept for 21 days at 3° C.
(2) ,, 21 ,, 1° C.

April 28th sowing (field):

(1) Grain kept for 14 days at 3° C.

Control samples of unvernalised grain of each variety were included in the sowings. These controls were treated similarly to the vernalised samples in that they were subjected to the preliminary soaking in germinating dishes for 24 hours before sowing. The vernalised and control samples were dibbed out in small adjacent plots of 100 grains each in the field sowings. In the glasshouse sowing about twelve plants were grown in each pot.

OBSERVATIONS.

The following observations apply only to the March 1st and March 8th sowings. The April 28th sowing is treated separately later.

(1) *Appearance of the plants above the ground.*

For convenience the appearance of the plants above the ground is termed "germination" in this paper. Table I gives the number of days taken by the treated samples to appear above the ground, and the acceleration due to vernalisation treatment.

Table I.

	March 1st (field)						March 8th (field)				March 8th (glasshouse)			
	14 days at 3° C.		7 days at 3° C.		14 days at 1° C.		21 days at 3° C.		21 days at 1° C.		21 days at 3° C.		21 days at 1° C.	
Variety	No. of days	Accel.	No. of days	Accel.	No. of days	Accel.	No. of days	Accel.	No. of days	Accel.	No. of days	Accel.	No. of days	Accel.
Wintergerste	19	4	19	4	19	4	17	2	18	1	9	5	11	3
Bocumer	19	4	20	3	19	4	18	2	17	3	10	5	10	5
H. spontaneum	20	4	—	—	—	—	—	—	—	—	—	—	—	—
Pryor's Chevallier	18	4	—	—	—	—	—	—	—	—	—	—	—	—
H. Haxtonii	17	6	—	—	—	—	—	—	—	—	—	—	—	—
Goldthorpe	19	4	—	—	—	—	17	3	—	—	8	6	—	—
Spratt-Archer	19	3	—	—	—	—	17	3	—	—	—	—	—	—
Swanneck	18	5	—	—	—	—	16	3	—	—	6	5	—	—
H. praecox	21	3	—	—	—	—	18	2	—	—	—	3	—	—
Big-wheat Barley	21	2	—	—	—	—	18	3	—	—	—	—	—	—
April Bearded	20	3	—	—	—	—	18	2	—	—	10	1	—	—
Joss IV	24	0	—	—	—	—	18	1	—	—	10	2	—	—
Yeoman II	24	1	—	—	—	—	18	1	—	—	9	2	—	—
Cambridge Rivett	24	2	—	—	—	—	18	1	—	—	8	4	—	—
Abundance	24	2	—	—	—	—	17	3	—	—	8	6	—	—
Argentine	24	2	—	—	—	—	18	2	—	—	—	—	—	—
Bountiful	23	3	—	—	—	—	17	3	—	—	8	4	—	
Marvellous	23	3	—	—	—	—	17	3	—	—	—	—	—	—
Grey Winter	21	4	—	—	—	—	17	3	—	—	9	5	—	—

This table requires little elaboration, but attention may be drawn to the more important points. In the March 1st sowing the only variety that did not show germination acceleration was the winter wheat variety Joss IV, while the spring barleys *H. Haxtonii* and Swanneck gave the greatest acceleration. It is of interest to notice that the excessively early spring barley, Pryor's Chevallier, showed as great an acceleration as the winter barleys. In the wheats, also, the spring variety, April Bearded, was more responsive than the winter varieties; but the spring oats were not affected so markedly as Grey Winter. The different treatments given to Bocumer and Wintergerste gave no conclusive results.

Germination acceleration was not as great in the March 8th field sowing in some varieties, but was slightly greater in others. The reason for this is difficult to see, because it would be expected that the longer vernalisation and the later sowing would stimulate greater differences in all cases even though there was only one week's difference in the sowing time. It is possible that the continued dry conditions affected the partially germinated treated grain more than the controls, resulting in a proportionally larger check to their growth.

Germination in the glasshouse was naturally much quicker than in the field, and with the exception of April Bearded, there were greater differences between the germination times of controls and vernalised samples. It should be noticed that spring varieties of barley and oats

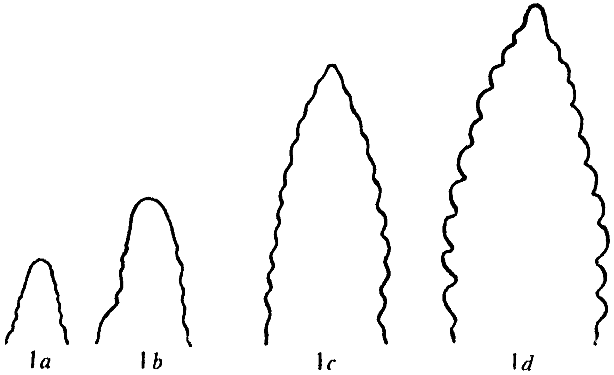

Fig. 1. Cambridge Rivett. 1 *a*, control at 78 days; 1 *b*, vernalised at 78 days; 1 *c*, control at 87 days; 1 *d*, vernalised at 87 days.

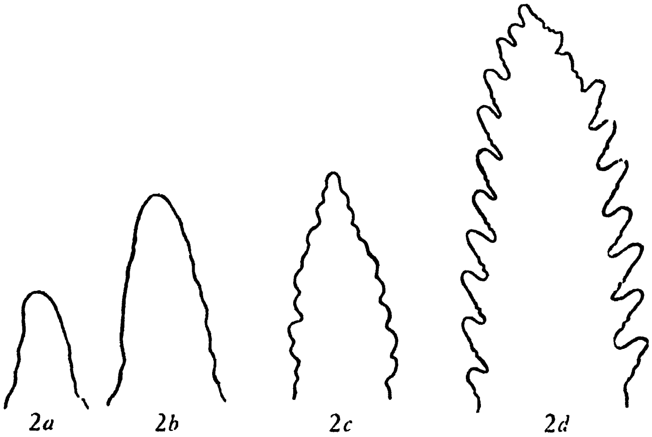

Fig. 2. Joss IV. 2 *a*, control at 78 days; 2 *b*, vernalised at 78 days; 2 *c*, control at 87 days; 2 *d*, vernalised at 87 days.

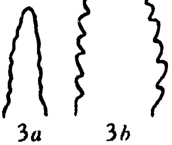

Fig. 3. Yeoman II. 3 *a*, control at 78 days; 3 *b*, vernalised at 78 days.

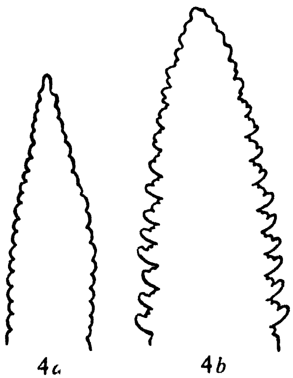

Fig. 4. Bocumer. 4 *a*, control at 78 days; 4 *b*, vernalised at 78 days.

(All magnifications approximately 17 times.)

are amongst those showing the greatest acceleration. In both the March 8th sowings the different treatments of Wintergerstc and Bocumer gave no conclusive results of differential action.

(2) *Juvenile response.*

After germination the effect of vernalisation could be seen in the juvenile habit and luxuriance of growth when the plants were 6–7 weeks old. In all barley varieties the vernalised samples were more luxuriant than the controls; and where the variety was of a procumbent or prostrate habit, vernalisation also had the effect of making this habit more erect. This was true of all sowings, but was more pronounced in the glasshouse sowing.

At this stage the only wheat variety to show vernalisation effect was Rivett, where the treated plants were more erect. In fact the glasshouse vernalised plants of Joss IV and Yeoman II were somewhat more procumbent than the controls.

All the oat varieties, with the exception of Grey Winter, are normally of a more or less erect habit. Grey Winter responded to the treatment by increasing erectness and luxuriance, while the other varieties showed greater luxuriance only.

(3) *Growing point development.*

Examination of the growing points of both winter and spring varieties at regular intervals showed that the vernalised plants had their growing points further developed than the controls. The growing points of the main tillers of sample plants were dissected out, and in all cases they showed greater size and differentiation in the treated plants. Observations were made first when the plants were nearly two months old, and four plants were pulled up and examined from each variety taken. This was repeated three times at 10-day intervals, until the plants had commenced shooting. No critical measurements were made, but the growing points were examined under the microscope, although in many cases the larger size of the growing points of the vernalised plants was easily discernible with the naked eye. (See text-figs. 1–4.)

The evidence of the stimulation to quicker development brought about by low temperature treatment can thus be augmented by a growing point investigation. How soon in the growth of the plant may differences be seen in the growing point is not known, and a more critical and detailed investigation of this point is needed.

(4) *Earing.*

Table II shows the acceleration in earing date due to vernalisation treatment. The columns headed "control" give the number of days taken by the controls to come into ear from sowing time. The columns headed "acceleration" give the acceleration in days due to the various treatment.

Table II.

	March 1st (field)				March 8th (field)			March 8th (glasshouse)				
	1	2	3	4	5	6	7	8	9	10	11	12
		Accel.				Accel.			Accel.			
Variety	Control	14 days at 3° C.	7 days at 3° C.	14 days at 1° C.	Control	21 days at 3° C.	21 days at 1° C.	Control	21 days at 3° C.	21 days at 1° C.	Col. 9–col. 6	Col. 8–col. 5
Wintergerste	93	3	2	4	90	5	5	98	14	14	9	8
Bocumer	101	6	2	4	97	6	7	104	15	15	9	7
H. spontaneum	88	3	—	—	—	—	—	—	—	—	—	—
H. praecox	95	3	—	—	89	2	—	94	7	—	5	5
H. Haxtonii	94	1	—	—	—	—	—	—	—	—	—	—
Big-wheat Barley	89	0	—	—	82	0	—	—	—	—	—	—
Swanneck	92	0	—	—	85	0	—	87	2	—	2	2
Pryor's Chevallier	83	0	—	—	—	—	—	—	—	—	—	—
Spratt-Archer	100	2	—	—	92	3	—	—	—	—	—	—
Goldthorpe	103	1	—	—	98	2	—	98	2	—	0	0
April Bearded	102	0	—	—	96	0	—	96	0	—	0	0
Joss IV	111	3	—	—	106	4	—	117	7	—	3	11
Yeoman II	109	2	—	—	105	4	—	119	10	—	6	14
Cambridge Rivett	112	2	—	—	106	3	—	124	16	—	13	18
Abundance	104	1	—	—	98	1	—	96	6	—	5	−2
Argentine	105	1	—	—	99	1	—	—	—	—	—	—
Bountiful	101	2	—	—	95	2	—	98	1	—	−1	3
Marvellous	103	2	—	—	98	2	—	—	—	—	—	—
Grey Winter	104	2	—	—	97	1	—	99	1	—	0	2

It can be seen from this table that the winter varieties of wheat and barley show the greatest acceleration of earing in all three sowings. The oat varieties do not respond in this way. In the March 1st sowing only the spring varieties Pryor's Chevallier, Swanneck, Big-wheat Barley, and April Bearded, failed to give earing acceleration. The varieties sown on March 8th in general confirm the March 1st results, but the winter varieties tended to show greater acceleration, with the exception of *H. praecox* and Grey Winter.

The varieties sown in the glasshouse are of especial interest. The winter varieties Wintergerste, Bocumer, Yeoman II and Cambridge Rivett showed much more pronounced earing acceleration than in the field sowings; while *H. praecox*, Joss IV and Abundance were similarly, though less markedly, affected. Grey Winter and Bountiful were peculiar in showing less response than in the field sowings. It is also worth noting that the early earing spring barley Swanneck showed a small earing acceleration in the glasshouse sowing, although it had been quite unresponsive in the field.

The different treatments given to Bocumer and Wintergerste were somewhat inconclusive. In the March 1st sowing there is a suggestion that vernalisation for 7 days was not as effective as for 14 days; while 3° C. appeared more effective for Bocumer than 1° C. It is probable that the longer exposure of 21 days masked any differential effect of temperature in the later sowings. Lyssenko claims that vernalisation is complete after 10 days, but that treatment is slower in acting when the temperature is allowed to approach 0° C.

April 28th sowing.

The sowing of winter cereal varieties as late as the end of April in this country normally results in failure to ear. It therefore seemed worth while to test the effect of vernalisation on winter varieties when sown as late as this.

The varieties chosen were Bocumer, Wintergerste, *H. spontaneum*, Yeoman II, Joss IV, Rivett, and Grey Winter. The treated samples were vernalised for 14 days at 3° C., and were dibbed out in the field with controls as in the previous sowings.

No acceleration in germination due to the vernalisation was apparent, nor was there any modification of the plants in the juvenile stages of growth. The first response to the treatment was in earing. At the end of the second week of July the vernalised plots of Bocumer, Wintergerste, and *H. spontaneum* began to ear irregularly. The controls of Bocumer and *H. spontaneum* showed one plant with elongated culms by the third week of September, while the Wintergerste control remained in the juvenile stage. Cambridge Rivett behaved in the same way, while Joss IV showed two earing plants in the vernalised plots at the beginning of September, and one earing plant in the control plot by the end of the second week of September. Yeoman II showed little vernalisation effect, both the control and the treated samples coming into ear at the beginning of the third week of July within a few days of one another. Grey Winter was completely unresponsive, both the control and treated plots earing at the beginning of July. Owing to the unevenness of earing in this late sowing, no attempt was made to take critical earing dates.

DISCUSSION.

The results of these preliminary experiments appear to show that low temperature treatment of the germinating grain of cereals results in a complete physiological stimulation of the developing plant, which may manifest itself as early in the life history as the appearance of the plants above the ground. It is true that one variety, *e.g.* Joss IV, failed to show this early growth acceleration, while other varieties were so little affected that it might be attributed to chance fluctuation in grain, or differences in the growing conditions. It might also be argued that the quicker appearance of the vernalised plants above the ground was due to their more advanced stage of development at planting. This might account for a 1-day difference, and it would be expected that every variety would behave similarly if this was the case. The differential response of the varieties, and the fact that some of the vernalised grain in any treated sample showed no signs of radicle extrusion when planted, while others had roots half an inch in length without affecting the evenness of the germination, suggest that any differences in the morphological development of the grain at planting will not account for the more rapid early growth of the vernalised plants.

The changes in the juvenile habit of the vernalised plants may also be taken as evidence of physiological stimulation. Vernalisation resulted in greater erectness of habit in the majority of varieties. It is interesting to notice that, under ordinary field conditions, conditions of growth which encourage rapid development also encourage a more erect habit of growth. Thus, a variety sown in the winter may have a very procumbent habit during the winter months when growth is slow. When the warmer weather of spring stimulates more rapid growth the habit of growth becomes considerably more erect. Further, a procumbent variety sown late in the spring generally assumes a more erect habit than when winter or early spring sown. Arguing from this evidence, and the greater luxuriance of growth, there is reason to conclude that vernalisation treatment has stimulated the early growth rate.

Concrete evidence in favour of early developmental stimulation could be obtained from examination of the growing points. The comparatively late stage when the growing points were examined in the present experiments show definite vernalisation stimulation. This was true even for the earliest earing spring variety Pryor's Chevallier which, although showing no earing acceleration, was stimulated to more rapid growing point development by the low temperature treatment.

When the effects of the grain pre-treatment on the early stages of the plant's development are considered in conjunction with the effect on the earing date, some interesting points may be noticed. Spring varieties that had shown marked germination or juvenile response, either failed to show any earing acceleration at all, or else were only slightly affected. Conversely, some winter varieties that had appeared completely, or very little, affected in the early stages, were among those showing maximum earing acceleration. Low temperature treatment can thus apparently affect the plant at different phenological periods of its development; the acceleration of earing, or "vernalising" effect, is only one aspect of this. Even though the low temperature does not "vernalise" it might stimulate in other ways, such as yield increase or increased resistance to disease.

In the varieties tested there was no simple correlation between earing date and the degree of earing acceleration due to grain pre-treatment. It is true that in general the earliest varieties gave the least, and the latest varieties the greatest, earing acceleration. But in the March 1st sowing the early earing winter varieties *H. praecox*, Wintergerste and *H. spontaneum* showed a greater acceleration than some of the later earing forms (Table II).

The very much more pronounced response to treatment in the glasshouse sowing suggests that the field sowings were too early to show the maximum effect of low temperature exposure. In Table III are given the varietal earing dates, and it can be seen that the controls of all varieties that gave the largest glasshouse acceleration in earing came

Table III.

	March 1st (field)				March 8th (field)			March 8th (glasshouse)		
Variety	Control	14 days at 3° C.	7 days at 3° C.	14 days at 1° C.	Control	21 days at 3° C.	21 days at 1° C.	Control	21 days at 3° C.	21 days at 1° C.
Wintergerste	2·6	30·5	31·5	29·5	6·6	1·6	1·6	14·6	31·5	31·5
Bocumer	10·6	4·6	8·6	6·6	13·6	7·6	6·6	20·6	5·6	4·6
H. spontaneum	27·5	24·5	—	—	—	—	—	—	—	—
H. praecox	4·6	1·6	—	—	5·6	3·6	—	10·6	3·6	—
H. Haxtonii	3·6	2·6	—	—	—	—	—	—	—	—
Big-wheat Barley	28·5	28·5	—	—	29·5	29·5	—	—	—	—
Swanneck	1·6	1·6	—	—	1·6	1·6	—	3·6	1·6	—
Pryor's Chevallier	23·5	23·5	—	—	—	—	—	—	—	—
Spratt-Archer	9·6	7·6	—	—	8·6	5·6	—	—	—	—
Goldthorpe	12·6	11·6	—	—	14·6	12·6	—	14·6	12·6	—
April Bearded	11·6	11·6	—	—	12·6	12·6	—	12·6	12·6	—
Joss IV	20·6	17·6	—	—	22·6	18·6	—	3·7	26·6	—
Yeoman II	18·6	16·6	—	—	21·6	17·6	—	5·7	25·6	—
Cambridge Rivett	21·6	18·6	—	—	22·6	19·6	—	10·7	24·6	—
Abundance	13·6	12·6	—	—	14·6	13·6	—	12·6	6·6	—
Argentine	14·6	13·6	—	—	15·6	14·6	—	—	—	—
Bountiful	10·6	8·6	—	—	11·6	9·6	—	14·6	13·6	—
Marvellous	12·6	10·6	—	—	14·6	12·6	—	—	—	—
Grey Winter	13·6	11·6	—	—	13·6	12·6	—	15·6	14·6	

into ear considerably earlier in the field than in the glasshouse. This suggests that the controls in the field were stimulated to earlier earing by the ordinary field temperature of an early spring sowing.

It is of interest to compare the differences in earing acceleration between the March 8th field and glasshouse sowings, with the differences in earing time of the controls under these two sets of conditions. These figures are given in Table II, columns 11 and 12, and it can be seen that there is a certain agreement between the two sets of figures in the barley and wheat varieties. Thus, in the five barley varieties tested, the differences between the earing acceleration in the field and in the glasshouse were 9, 9, 5, 2, and 0 days respectively. The differences in the earing date between the controls of these varieties under the two sets of conditions were 8, 7, 5, 2, and 0 days respectively. The greater earing acceleration of these varieties in the glasshouse was due almost entirely to the later earing date of the glasshouse controls compared with the field controls, there being little difference between the glasshouse and field vernalised plants of any variety (see Table III).

This relationship does not hold for the wheat varieties because in all cases (except the entirely unaffected April Bearded) the treated samples came into ear considerably later in the glasshouse than in the field (Table III). Thus, although there is a parallel in the magnitude of the difference in the earing dates of the field and glasshouse controls, and the differences in earing acceleration under the two sets of conditions, there is not the close relationship that was seen in the barley varieties (Table II, cols. 11 and 12). Because the vernalised plants in the glasshouse eared later than the vernalised plants in the field, the earing acceleration in the glasshouse was not as large as it otherwise would have been. The earlier earing of the vernalised field plots, compared with the vernalised glasshouse plants, was possibly due to a further vernalising effect of the somewhat low soil temperatures in the field. This supplemented the artificial vernalisation, which apparently was not adequate for bringing about the maximum earing acceleration possible under the particular growing conditions.

The behaviour of the two oat varieties Abundance and Bountiful is difficult to explain. Both the vernalised and control samples of Abundance came into ear earlier in the glasshouse than in the field. The greater glasshouse acceleration in earing is due almost entirely to the greater earliness of the vernalised plants. This is an entirely anomalous position compared with the wheat and barley varieties. The variety Bountiful behaved very much like the wheat varieties with regard to the later earing dates in the glasshouse, but it was peculiar in that the glasshouse acceleration was smaller than that in the field.

In spite of the irregular behaviour of these two oat varieties, the general results of these observations uphold Lyssenko's main contention that the vegetative phase of long-day plants can be shortened by low temperature seed pre-treatment. The method of vernalisation used for the wheat, barley and oats in the present experiments was identical in its essentials to that employed by Lyssenko for wheat only, and the results show that the same method is as effective for winter barley varieties. It is equally obvious, however, that the reaction to vernalisation treatment is a varietal character which may manifest itself in other ways than reproductive stimulation.

Apart from the extreme academic interest of the physiological aspect of vernalisation, there is a direct practical consideration of agricultural importance. Vernalisation seed pre-treatment appears to offer the possibility of artificially inducing early flowering on an economic scale. Lyssenko has proved its practical value for wheat on the Russian Steppes. In this country the application appears to lie more with horticultural crops where stimulation to early development, early flowering, or flowering out of season, would have far-reaching effects on the growing of market garden crops.

In addition to the above consideration, vernalisation research has already helped to a better understanding of some physiological problems. Take, for example, the important question of "earliness" in crop plants. Lyssenko maintains that earliness in long-day plants is governed primarily by temperature conditions. McKinney and Sando (4) on the other hand claim that "within limits temperature and daily-photoperiod compensate for each other in their influence on earliness", and they bring forward evidence to show that the effect of low temperature seed-pre-treatment is modified by light and temperature conditions during subsequent growth. In the present paper the modifying effect of temperature during early growth on the action of low temperature treatment has been shown, but critical experiments on the relative effects of light and temperature on earliness have yet to be done.

SUMMARY.

1. Low temperature grain pre-treatment of some varieties of wheat, barley, and oats, has resulted, in most cases, in a stimulation to early development of the plant with a modification of the juvenile habit.

2. In addition to early developmental stimulation, winter varieties of barley and wheat also showed marked acceleration in the date of earing. Spring varieties either failed to show this earing acceleration or else were but slightly affected.

3. Glasshouse culture during the first few months of growth resulted, in most varieties, in a greater response to the grain pre-treatment. It is suggested that this was due to the absence of low temperatures in the glasshouse.

4. Response to low temperature pre-treatment is a varietal character.

The writer wishes to acknowledge the kindness of F. Kidd, Esq., M.A., by whose courtesy accommodation was made available in the Low Temperature Research Station.

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(*Received October* 30*th*, 1934.)

THE USE OF "DIFFERENTIAL REGRESSION" IN ANALYSIS OF VARIANCE.

By WALTER A. HENDRICKS, *Junior Biologist.*
(*Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C.*)

The increasing popularity of the methods of analysis of variance and covariance, as described by Fisher (1932), has led to the application of those methods to problems to which they are not appropriate without modification. Yates (1933) has called attention to modifications which may be necessary for the treatment of data involving disproportionate frequencies. It is the purpose of the present paper to point out a modification which must sometimes be made in the method of correcting the entries in an analysis of variance table for the influence of a correlated variate.

Consider the data in Table I. These data were obtained in the Nutrition Laboratory at the U.S. Animal Husbandry Experiment Farm, Beltsville, Md., and relate to the feed consumption and gains in live weight of fourteen white rats during an experimental period of eight weeks. The nutritive values of two diets were being compared.

Table I. *Feed consumption and gains in live weight of rats on two different diets.*

Diet No.	Rat No.	Feed consumed gm.	Gains in live weight gm.	Diet No.	Rat No.	Feed consumed gm.	Gains in live weight gm.
1	1	174	0	2	8	398	110
	2	205	25		9	262	54
	3	191	13		10	273	47
	4	250	50		11	297	53
	5	224	27		12	234	41
	6	233	18		13	348	81
	7	294	45		14	314	46

Crampton (1934) has suggested that such data be treated by the methods of analysis of variance and covariance as described by Fisher (1932). In this procedure the influence of differences in feed consumption is supposed to be removed from the variability of the gains in live weight by means of a net regression equation. Table II presents the results of applying this method of analysis to the data in Table I. The details of computation are so well known that no discussion of them is required here.

Table II. *Analysis of variance of gains in live weight corrected for differences in feed consumption. (Ordinary variance and covariance analysis.)*

Source of variability	Degrees of freedom	Sum of squares	Mean square
Between diets	1	83·13	83·13
Within diets	11	1158·41	105·31
Total	12	1241·54	—

It is evident from a consideration of the nature of the data in Table I that the method of analysis leading to the results given in Table II may not be appropriate. The analysis was made on the assumption that the regression of gain in live weight on amount of feed consumed was the same regardless of which diet the rats received. If the two diets which were being compared differed with respect to nutritive value, this assumption is not justifiable. A unit difference in feed consumption should have a greater effect on the magnitude of a gain in live weight in the case of a good diet than in the case of a poor one. Therefore, it seems desirable to calculate a separate regression coefficient for each diet.

The "sums of squares" and "sums of products" for feed consumption and gains in live weight may be calculated in the usual manner. However, when recording the sums of squares and products "within diets", the respective values are tabulated separately for each diet. Two regression coefficients may be calculated from these two sets of values. In the present example the values of the regression coefficients are as follows:

$$b_1 = 0{\cdot}378078, \quad b_2 = 0{\cdot}405568.$$

By means of these regression coefficients the observed gains in live weight may be adjusted to a common feed consumption in the customary manner, with the single difference that a different regression coefficient is employed in adjusting the gains for each diet.

In actual practice it is not necessary to apply the corrections to the individual gains, although the work cannot be simplified to the same extent as in the ordinary variance and covariance analysis. In order to obtain the analysis of variance of the adjusted gains, it is necessary to know only the total of the adjusted gains for each diet and the sum of the squares of all of the adjusted gains.

Let x denote the observed feed consumption of a single rat and let

$\bar{x}$ denote the average feed consumption of all of the rats. Let $x' = x - \bar{x}$. The sum of the deviations of the observed values of x from $\bar{x}$ may be calculated separately for each diet by means of the formulas:

$$\left.\begin{aligned}(x')_1 &= (x)_1 - n_1\bar{x}\\ (x')_2 &= (x)_2 - n_2\bar{x}\end{aligned}\right\} \quad \ldots\ldots(1),$$

in which the parentheses indicate summation, the subscripts indicate the diets to which the given sums correspond, and n_1 and n_2 are the numbers of rats which received the respective diets.

Let y be the observed gain in live weight, made by a single rat, which corresponds to the feed consumption, x. The total of the adjusted gains for each diet is given by the formulas:

$$\left.\begin{aligned}(Y)_1 &= (y)_1 - b_1(x')_1\\ (Y)_2 &= (y)_2 - b_2(x')_2\end{aligned}\right\} \quad \ldots\ldots(2),$$

in which Y represents the adjusted gain in live weight for a single rat and the parentheses and subscripts have the same significance as in equations (1).

The sum of the squares of the adjusted gains is given by the formula:

$$(Y^2) = (y^2) - 2b_1(x'y)_1 - 2b_2(x'y)_2 + b_1^2(x'^2)_1 + b_2^2(x'^2)_2 \ldots\ldots(3),$$

in which

$$\left.\begin{aligned}(x'y)_1 &= (xy)_1 - \bar{x}(y)_1\\ (x'y)_2 &= (xy)_2 - \bar{x}(y)_2\\ (x'^2)_1 &= (x^2)_1 - 2\bar{x}(x)_1 + n_1\bar{x}^2\\ (x'^2)_2 &= (x^2)_2 - 2\bar{x}(x)_2 + n_2\bar{x}^2\end{aligned}\right\} \quad \ldots\ldots(4).$$

The analysis of variance for the adjusted gains may be obtained in the usual manner from the totals of the adjusted gains for each diet and the sum of the squares of all of the adjusted gains. The results of the analysis are given in Table III.

Table III. *Analysis of variance of gains in live weight corrected for differences in feed consumption. (Method of differential regression.)*

Source of variability	Degrees of freedom	Sum of squares	Mean square
Between diets	1	95·36	95·36
Within diets	10	1153·62	115·36
Total	11	1248·98	—

A comparison of Tables II and III does not reveal any striking differences between the results of the two different methods of analysis. The variance "between diets" was increased by the use of two regression coefficients in the analysis, but because of the small amount of data and the small difference between the numerical values of the regression coefficients for the two diets, the loss of the additional degree of freedom "within diets" caused the variance "within diets" to increase almost as much as the variance "between diets".

The value of the principle of differential regression may be more impressively demonstrated by a study of the effects of some vitamin supplements in the diets of laying hens upon the hatchability of their eggs. This study was made by members of the staff of the Poultry Nutrition Laboratory at Beltsville. Six lots of chickens were used to obtain the necessary data. Two different vitamin supplements were fed to four lots of birds in such a manner that two lots received each supplement. The remaining two lots of birds received no special supplements and served as controls. Eggs from each lot of birds were incubated at regular intervals during the first year of the experiment to test the effects of the experimental treatments upon the hatchability of the eggs. Eight such incubation trials were conducted. The number of fertile eggs set varied from lot to lot and from period to period. The data obtained are given in Table IV.

Table IV. *Number of fertile eggs set and number of chicks hatched in each incubation trial.*

	Lot 1		Lot 2		Lot 3		Lot 1*a*		Lot 2*a*		Lot 3*a*	
Trial	Eggs	Chicks	Eggs	Chicks	Eggs	Chicks	Eggs	Chicks	Eggs	Chicks	Eggs	Chicks
1	37	21	22	20	15	13	25	9	23	15	21	7
2	28	4	27	1	46	18	11	1	21	3	69	42
3	28	19	5	4	54	47	15	9	3	1	55	43
4	42	19	6	2	62	31	24	12	—	—	62	25
5	74	47	1	1	54	39	33	15	—	—	77	46
6	56	45	—	—	43	36	18	13	—	—	39	33
7	50	23	11	4	46	27	25	6	7	4	39	25
8	38	18	5	2	38	15	12	2	3	2	46	24

The choice of a suitable unit to be used in the statistical treatment of such data involves some difficulty. The percentage hatch, calculated for each lot of birds for each incubation trial, is not a satisfactory unit if the data are to be studied by analysis of variance, since the number of fertile eggs set was not constant. In the opinion of the author of this paper, the method of analysis of variance should be applied to the numbers of chicks hatched after adjusting these values to the same numbers of fertile eggs set by the method of differential regression.

The variability in the adjusted numbers of chicks hatched may be separated into the components, "between treatments", "between lots with the same treatment", and "within lots". The adjustment of the numbers of chicks hatched should be made by means of six regression coefficients corresponding to the six sub-classes or lots. The numerical values of these regression coefficients are as follows:

$b_1 = 0{\cdot}834387$, $b_{1a} = 0{\cdot}502299$,
$b_2 = 0{\cdot}313589$, $b_{2a} = 0{\cdot}413223$,
$b_3 = 0{\cdot}594450$, $b_{3a} = 0{\cdot}584255$.

The analysis of variance of the adjusted numbers of chicks hatched is given in Table V. The details of computation are similar to those involved in the analysis summarised in Table III, except that the variance "between treatments" was calculated by Yates' method of "weighted squares of means" because of the disproportionate numbers of observations in the subclasses.

Table V. *Analysis of variance of numbers of chicks hatched corrected for differences in numbers of fertile eggs set. (Method of differential regression.)*

Source of variability	Degrees of freedom	Sum of squares	Mean square
Between treatments	2	462·11	231·05
Between lots with the same treatment	3	18·20	6·07
Within lots	32	1581·38	49·42
Total	37	—	—

If the numbers of chicks hatched are adjusted by means of a single regression coefficient, as in the ordinary variance and covariance analysis, the misleading results given in Table VI are obtained.

Table VI. *Analysis of variance of numbers of chicks hatched corrected for differences in numbers of fertile eggs set. (Ordinary variance and covariance analysis.)*

Source of variability	Degrees of freedom	Sum of squares	Mean square
Between treatments	2	96·94	48·47
Between lots with the same treatment	3	18·86	6·29
Within lots	37	1738·24	46·98
Total	42	—	—

SUMMARY.

The application of a principle of "differential regression", which is of value in the statistical treatment of some types of experimental data, is illustrated. Briefly stated, the principle involves the use of a separate regression coefficient for each subclass of observations when correcting

these observations for the influence of variability in a correlated variate, instead of the use of an average net regression coefficient as employed in the usual variance and covariance analysis.

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(*Received September 1st*, 1934.)

A GENETIC ANALYSIS OF THE SEED CHARACTERS WRINKLED, DIMPLED AND SMOOTH IN *PISUM*.

BY J. W. HADFIELD AND R. A. CALDER.
(*Plant Research Station, Palmerston North, New Zealand.*)

(With Plates IV, V.)

I. INTRODUCTION.

IN the breeding of field and garden peas for commercial purposes the shape or surface form of the seed may be regarded as a matter of considerable importance. The writers, having failed to correlate the observed results of their crossings with those recorded by other workers, have reinvestigated the genetic constitution of the three seed forms, wrinkled, dimpled, and smooth. The results of these investigations are now offered.

The wrinkled and smooth forms.

Wrinkled seed is generally found associated with the more popular garden varieties such as Stratagem, Daisy, Alderman, and Gradus. On the other hand smooth-seeded varieties such as Blue Prussian, White Ivory, and Victoria are classed as field peas.

The dimpled form.

In appearance, but in this respect only, the dimpled form may be regarded as being intermediate between the wrinkled and the smooth. Trade marrowfats, such as Harrison's Glory, garden varieties such as Day's Early Sunrise and Pride of the Market, and the field varieties Partridge and Dun, are all dimple-seeded.

Most dimple-seeded varieties when grown at this Station produce varying proportions of "smooth"[1] seeds. Investigations instituted by the writers to discover the nature and origin of "smooth" seeds in Harrison's Glory have been reported elsewhere,[2] and are summarised here because of the direct and important bearing they have upon the discussion which is to follow.

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[1] To avoid confusion genotypically dimpled seeds that are phenotypically smooth are referred to in the text within inverted commas, thus "smooth" or in the tables as "S."

[2] *N.Z. J. Sci. Tech.* (1933). **14**, No 4.

(1) "Smooth" seed yielded in its progeny no greater proportion of "smooth" seeds than did the dimpled, and, when crossed with wrinkled and true smooth, "smooth" and dimpled proved to be genetically similar.

(2) "Smooth" seed was found more commonly in the upper pods or in those arising on lateral branches. When both "smooth" and dimpled seeds occurred on the same plant, there was to be found a gradation in shape from dimpling in the lower pods to "smooth" in the upper pods (Pl. I, fig. 1). All seeds in any one pod exhibited the same form. There was no segregation by seed in the pod.

(3) Seasonal and nutritional factors had a direct bearing on the occurrence of "smooth" seeds.

From this evidence it may be concluded that dimpling is a highly modifiable character and that genetically there is no difference between the "smooth" and dimpled seeds arising on dimple-seeded varieties. The degree of modification may be extreme. At this Station in 1930–1 Harrison's Glory contained 30 per cent. of "smooth" seeds, in 1931–2 8 per cent., and in 1932–3 20 per cent.

Expression of seed forms in transmission.

Although each of the three characters exerts a determining influence affecting the shape of the seed and therefore that of the cotyledons, yet the expression of each in a plant or generation is not observable at the same stage of development. Making due allowance for dominance and epistasis, the wrinkled and smooth characters will, if brought in by means of crossing, affect immediately the appearance of the F_1 seed and will segregate by seed on the F_1 plant. Dimpling if brought in by means of crossing does not influence the form of the F_1 seed of that cross. Its expression is not apparent till the F_2 seed has ripened, and segregation, which occurs by plant, is not observed until the F_2 plants have reached maturity. Wrinkled and smooth may be regarded as being transmitted as cotyledon characters, and dimpling as being transmitted as a vegetative character in a manner similar in this respect to that of flower or seed-coat colour.

Epistasis.

Were it possible to deal with the factors for smooth and wrinkled in the absence of dimpling, segregation would no doubt be monofactorial, giving the ratio 3 : 1 with smooth dominant. All the wrinkled varieties with which the writers have worked are proved, however, to contain dimpling in association with wrinkling. Since a seed cannot at the one time exhibit wrinkling and dimpling, it follows that such genotypically wrinkled-dimpled seeds are all phenotypically wrinkled and remain constant. Wrinkling is therefore epistatic to dimpling. Similarly dimpling is epistatic to smooth.

Suggested genetic formulae.

S. J. Wellensiek in his "Genetic monograph on *Pisum*," *Bibliographia Genetica*, vol. **2**, has given a full review of the literature dealing with this subject published since 1900 when Mendel's work was rediscovered. Such references as are made here to the work of other investigators will be found in Wellensiek's review.

The genetic analysis of the dimpled seed form is chiefly due to Tschermak, Lock, Bateson, and White, summarising which Wellensiek, on White's evidence, gives the following genetic formulae, using the symbols $\mathbf{RR}$ = smooth, $\mathbf{rr}$ = wrinkled, $\mathbf{L_1L_1L_2L_2}$ = dimpled in which $\mathbf{L_1}$ is either coupled with, or identical to, a ground factor for flower colour $\mathbf{A_1}$:

$\mathbf{L_1L_1l_2l_2RR}$ = smooth seed, coloured flower.
$\mathbf{l_1l_1L_2L_2RR}$ = smooth seed, white flower.
$\mathbf{L_1L_1L_2L_2RR}$ = dimple seed, coloured flower.
$\mathbf{l_1l_1L_2L_2rr}$ = wrinkled seed, white flower.

Wellensiek concludes: "Though White's conception appears to be highly plausible, further confirmation must be looked for, because the ratios, as far as they were mentioned, don't always agree as one would wish."

These genetic formulae do not account for the results obtained by the present writers, and they would offer instead the following as a sufficient explanation of all results observed from the crossing of commercial varieties:

$\mathbf{l_1l_1l_2l_2RR}$ = smooth.
$\mathbf{L_1L_1L_2L_2RR}$ = dimpled.
$\mathbf{L_1L_1L_2L_2rr}$ = wrinkled as found in most commercial wrinkled-seeded varieties.

$$\left.\begin{array}{r}\mathbf{l_1l_1L_2L_2rr}\\ \text{or }\ \mathbf{L_1L_1l_2l_2rr}\end{array}\right\} = \text{wrinkled as shown in at least one commercial wrinkle-seeded variety.}$$

Furthermore it is found that either $\mathbf{L_1}$ or $\mathbf{L_2}$ in either a heterozygous or a homozygous condition associated with $\mathbf{RR}$ results in dimpling, and such dimpling is epistatic to smooth.

II. SMOOTH $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ WRINKLED[1] (Pl. IV, fig. 2).

In this cross Mendel observed smooth to be dominant in F_1, and segregation in F_2 to be 3 S : 1 W. By means of back-crosses Mendel pointed out more definitely the monofactorial nature of this cross, and many other observers have corroborated these results.

The writers, on the contrary, have found in every case:

Wrinkled × Smooth $= F_1$ dimpled seed.
Smooth × Wrinkled $= F_1$ smooth seed.

Assuming the constitution of wrinkled to be $\mathbf{L_1L_1L_2L_2rr}$ and smooth to be $\mathbf{l_1l_1l_2l_2RR}$ and smooth $\mathbf{RR}$ to be dominant to wrinkled $\mathbf{rr}$, then:

$$\mathbf{L_1L_1L_2L_2rr} \times \mathbf{l_1l_1l_2l_2RR} = F_1\ \mathbf{L_1l_1L_2l_2Rr}\ \text{(dimpled)}.$$

It might be expected that the form of the F_1 seed would be determined by **Rr**, but since this seed is borne on a wrinkle seeded parent carrying the factors for dimpling $\mathbf{L_1L_2}$, and dimpling is epistatic to smooth, then **Rr** (smooth) cannot be expressed and the observed result is dimpling.

The crosses recorded in Table I prove this point; the appearance of a few "smooth" or nearly "smooth" seeds may be regarded here, and in all later results, as being due to modification of the dimpled character. (S = smooth. "S" = "smooth," that is proved to be genotypically dimpled. D = dimpled. W = wrinkled.)

Table I.

	Appearance of F_1 seed when ♂ parent is smooth-seeded	
Wrinkle-seeded ♀ parent	Smooth-seed ♂ parent, Blue Prussian	Smooth-seed ♂ parent, Victoria
Admiral Beatty	11 D + 6 almost "S"	14 D
Senator	19 D	9 D
American Wonder	8 D	3 D
Lord Chancellor	12 D	8 D
Daisy	10 D	2 D
English Wonder	6 D	—
Greenfeast, 1931–2*	32 D	—
Greenfeast, 1932–3*	13 almost "S"	20 almost "S"
Great Crop†	18 D	—
Stratagem	11 D + 1 "S"	—
		White Ivory ♂
Stratagem	—	24 D

* Greenfeast is a synonym for Lincoln.
† Great Crop is a synonym for Yorkshire Hero.

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[1] In all crosses the first named is the ♀ parent.

The reciprocal cross.

On the other hand in the reciprocal cross Smooth × Wrinkled, although the F_1 seed is necessarily again of the same constitution, yet it is borne on a mother plant in which the factors for dimpling are absent. Therefore **Rr** is both effective and observed and the resulting F_1 seed is smooth.

The crosses recorded in Table II prove this point.

Table II.

	Appearance of F_1 seed when ♀ parent is smooth-seeded	
Wrinkle-seeded ♂ parent	Smooth-seed ♀ parent, Blue Prussian	Smooth-seed ♀ parent, Victoria
Admiral Beatty	9 S	7 S
Senator	5 S	6 S
American Wonder	8 S	2 S + 3 slightly D
Lord Chancellor	6 S	1 slightly D
Daisy	16 S	9 S
English Wonder	11 S	—
Greenfeast, 1931–2	24 S	—
Greenfeast, 1932–3	10 S	10 S
Stratagem	8 S	16 S

F_2 seed arising on F_1 plants of Smooth $\underset{\leftarrow}{\overset{\rightarrow}{\times}}$ Wrinkled.

The Smooth $\underset{\leftarrow}{\overset{\rightarrow}{\times}}$ Wrinkled have in either case given the same result in F_2, namely a segregation by seed on the plant of 3 D : 1 W.

The explanation offered is that in $\mathbf{L_1l_1L_2l_2Rr} \times \mathbf{L_1l_1L_2l_2Rr}$ the **Rr** will segregate into 1 **RR** : 2 **Rr** : 1 **rr** and all these are borne on mother plants $\mathbf{L_1l_1L_2l_2Rr}$ which carry both factors for dimpling in a heterozygous form. The **RR** and **Rr** cannot be expressed on such plants, hence they remain dimpled. On the other hand wrinkling being epistatic to dimpling the **rr** is expressed as wrinkled. Thus the observed result is 3 dimpled to 1 wrinkled, instead of 3 smooth to 1 wrinkled, the dimpled carrying **RR** or **Rr** and the wrinkled **rr**.

Results confirming this are recorded in Table III.

In Table III the F_2 seed has been classified as "smooth," dimpled, and wrinkled. The totals show a close approximation to the 1 : 2 : 1 ratio and might lead to the supposition that "smooth" is the expression of the homozygous **RR** in Smooth × Wrinkled. It is suggested that this ratio is purely fortuitous. There is a gradation in shape from "smooth" to dimpling which makes it virtually impossible for two independent observers to come to an exact agreement as to what proportion should constitute the "smooth" and dimpled class respectively. Secondly, although in the totals the dimpled to "smooth" ratio is practically 2 : 1, this does not hold in the individual crosses which show considerable variation. Finally the "smooth" and dimpled F_2 seed from certain of the crosses has been sown separately and the evidence afforded by the segregation of the progenies has proved that "smooth" and dimpled are not genetically dissimilar.

Table III. *Surface form of* F_2 *seed on* F_1 *plants of* *Wrinkled* $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ *Smooth.*

Parents				1931/2	1932/3 F_2 seed on F_1 plants Numbers appearing in each group		
♀ parent	Seed form	♂ parent	Seed form	F_1 on parent plant Seed form	"Smooth"	Dimpled	Wrinkled
Stratagem	W	White Ivory	S	Dimpled	270	557	281
Great Crop 1	,,	Blue Prussian	,,	,,	120	345	154
Great Crop 2	,,	Blue Prussian	,,	,,	146	602	264
Greenfeast	,,	Blue Prussian	,,	,,	608	746	441
Blue Prussian	S	Greenfeast	W	Smooth	357	538	291
				Totals...	1501	2788	1431

Sum of "S" + D to W.. 4289 : 1431

Ratio "S" + D to W.. 2·99 : 1

Further confirmatory evidence of segregation in F_2 was obtained in 1933–4, but no attempt was made to separate dimpled from "smooth."

Table IV. *Surface form in* F_2 *seed of some* *Wrinkled* $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ *Smooth crosses,* 1933–4.

Parents				F_2 seed on F_1 plants	
♀ parent	Seed form	♂ parent	Seed form	Dimpled + "Smooth"	Wrinkled
Victoria	S	Lord Chancellor	W	120	41
Lord Chancellor	W	Crown	S	246	76
Victoria	S	Daisy	W	202	65
Daisy	W	Victoria	S	68	24
American Wonder	W	Victoria	S	91	34
Greenfeast	W	Victoria	S	162	50
		Totals		889	290
		Ratio of D + "S" to W			3·06 : 1

F_3 *seed arising on* F_2 *plants of* *Wrinkled* $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ *Smooth.*

The most satisfactory way of expressing this is by means of a checkerboard composed of squares which, individually, define the genetic con-

Diagram showing constitution of F_2 plants and expected segregation on same of F_3 seed from Smooth $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ Wrinkled.

Smooth × Wrinkled Wrinkled × Smooth

$l_1l_1l_2l_2RR$ $L_1L_1L_2L_2rr$ $L_1L_1L_2L_2rr$ $l_1l_1l_2l_2RR$

F_1 plants: $L_1l_1L_2l_2Rr$.

F_2 plants: Constitution below showing segregation of F_3 seed.

	RL_1L_2	RL_1l_2	Rl_1L_2	Rl_1l_2	rL_1L_2	rL_1l_2	rl_1L_2	rl_1l_2
RL_1L_2	RL_1L_2 RL_1L_2 D	RL_1l_2 RL_1L_2 D	Rl_1L_2 RL_1L_2 D	Rl_1l_2 RL_1L_2 D	rL_1L_2 RL_1L_2 3D : 1 Wr	rL_1l_2 RL_1L_2 3 D : 1 Wr	rl_1L_2 RL_1L_2 3 D : 1 Wr	rl_1l_2 RL_1L_2 3 D : 1 Wr
RL_1l_2	RL_1L_2 RL_1l_2 D	RL_1l_2 RL_1l_2 D	Rl_1L_2 RL_1l_2 D	Rl_1l_2 RL_1l_2 D	rL_1L_2 RL_1l_2 3 D : 1 Wr	rL_1l_2 RL_1l_2 3 D : 1 Wr	rl_1L_2 RL_1l_2 3 D : 1 Wr	rl_1l_2 RL_1l_2 3 D : 1 Wr
Rl_1L_2	RL_1L_2 Rl_1L_2 D	RL_1l_2 Rl_1L_2 D	Rl_1L_2 Rl_1L_2 D	Rl_1l_2 Rl_1L_2 D	rL_1L_2 Rl_1L_2 3 D : 1 Wr	rL_1l_2 Rl_1L_2 3 D : 1 Wr	rl_1L_2 Rl_1L_2 3 D : 1 Wr	rl_1l_2 Rl_1L_2 3 D : 1 Wr
Rl_1l_2	RL_1L_2 Rl_1l_2 D	RL_1l_2 Rl_1l_2 D	Rl_1L_2 Rl_1l_2 D	Rl_1l_2 Rl_1l_2 S	rL_1L_2 Rl_1l_2 3 D : 1 Wr	rL_1l_2 Rl_1l_2 3 D : 1 Wr	rl_1L_2 Rl_1l_2 3 D : 1 Wr	rl_1l_2 Rl_1l_2 3 S : 1 Wr
rL_1L_2	RL_1L_2 rL_1L_2 3 D : 1 Wr	RL_1l_2 rL_1L_2 3 D : 1 Wr	Rl_1L_2 rL_1L_2 3 D : 1 Wr	Rl_1l_2 rL_1L_2 3 D : 1 Wr	rL_1L_2 rL_1L_2 Wr	rL_1l_2 rL_1L_2 Wr	rl_1L_2 rL_1L_2 Wr	rl_1l_2 rL_1L_2 Wr
rL_1l_2	RL_1L_2 rL_1l_2 3 D : 1 Wr	RL_1l_2 rL_1l_2 3 D : 1 Wr	Rl_1L_2 rL_1l_2 3 D : 1 Wr	Rl_1l_2 rL_1l_2 3 D : 1 Wr	rL_1L_2 rL_1l_2 Wr	rL_1l_2 rL_1l_2 Wr	rl_1L_2 rL_1l_2 Wr	rl_1l_2 rL_1l_2 Wr
rl_1L_2	RL_1L_2 rl_1L_2 3 D : 1 Wr	RL_1l_2 rl_1L_2 3 D : 1 Wr	Rl_1L_2 rl_1L_2 3 D : 1 Wr	Rl_1l_2 rl_1L_2 3 D : 1 Wr	rL_1L_2 rl_1L_2 Wr	rL_1l_2 rl_1L_2 Wr	rl_1L_2 rl_1L_2 Wr	rl_1l_2 rl_1L_2 Wr
rl_1l_2	RL_1L_2 rl_1l_2 3 D : 1 Wr	RL_1l_2 rl_1l_2 3 D : 1 Wr	Rl_1L_2 rl_1l_2 3 D : 1 Wr	Rl_1l_2 rl_1l_2 3 S : 1 Wr	rL_1L_2 rl_1l_2 Wr	rL_1l_2 rl_1l_2 Wr	rl_1L_2 rl_1l_2 Wr	rl_1l_2 rl_1l_2 Wr

Ratio of the occurrence and the appearance of F_3 seed:
30 (3 D : 1 Wr); 15 D; 2 (3 S : 1 Wr); 1 S; 16 Wr.

stitution of the 64 possible combinations in F_2, and within which is noted also the shape or form of the F_3 seed that will be borne by a plant of that particular constitution. If F_2 seed is sown in the proportion of

3 dimpled : 1 wrinkled, the theoretical result should be 64 possible combinations bearing F_3 seed as follows:

A. Arising from the sowing of (16) F_2 wrinkled seed:
16 plants bearing all wrinkled F_3 seed.

B. Arising from the sowing of (48) F_2 dimpled seed:
30 plants bearing F_3 seed in the ratio of 3 D : 1 W.
15 „ „ that is all dimpled.
2 „ „ in the ratio of 3 S : 1 W.
1 plant „ „ that is all smooth.

Field results confirmed this expectation, the wrinkled F_2 seed remaining constant and the F_2 dimpled seed giving the results shown in Table V.

Table V. *Form of F_3 seed arising on F_2 plants in Wrinkled $\underset{\leftarrow}{\overset{\rightarrow}{\times}}$ Smooth when the wrinkled variety contains two factors for dimpling and only the dimpled F_2 seed is sown.*

	Number of F_2 plants upon which the forms arise		
F_3 seed forms	Observed number	Observed ratio	Expected ratio
3 D : 1 W	245	29·3	30
All D	121	14·4	15
3 S : 1 W	19	2·3	2
All S	17	2·0	1

Back-crosses.

In support of the suggested constitution of the smooth and wrinkled respectively results of the following back-crosses are given of W × (S × W) and W × (W × S). In either case the expectation is that $\mathbf{L_1L_1L_2L_2rr} \times \mathbf{L_1l_1L_2l_2Rr}$ will give equal numbers of dimpled and wrinkled seed. Actual results were 48 dimpled and 59 wrinkled seeds, the expectation being 53·5 of each.

Wrinkled varieties of the constitution $\mathbf{l_1l_1l_2l_2rr}$.

In wrinkled $\underset{\leftarrow}{\overset{\rightarrow}{\times}}$ smooth one-quarter of the F_2 plants bear wrinkled F_3 seed. Of these wrinkle-seeded plants one is a pure recessive $\mathbf{l_1l_1l_2l_2rr}$. If therefore the wrinkle-seeded F_2 plants are crossed with pollen from a smooth-seeded plant, the result should be in the ratio of 15 crosses resulting in dimpled seed, to 1 resulting in smooth seed. On 36 wrinkle-seeded F_2 plants crosses of this nature were made; 34 resulted in dimpled seed, and 2 resulted in smooth seed. The observed ratio of 17 : 1 is very close to the expected ratio of 15 : 1.

It is suggested that those investigators, who have observed Wrinkled × Smooth to result in smooth F_1 seed, either have not recognised the distinction between dimpled and smooth seeds, or have used a wrinkled variety of the constitution $\mathbf{l_1l_1l_2l_2rr}$. This is not regarded as impossible despite the fact that the writers have not so far discovered wrinkling of that constitution in the commercial varieties available at the present day.

Wrinkled seed of the constitution $\mathbf{l_1l_1L_2L_2rr}$.

At least one commercial variety, namely Greenfeast (synonym Lincoln), contains apparently but one factor for dimpling.

Granted such a constitution as $\mathbf{l_1l_1L_2L_2rr}$, the F_2 seed would consist of 3 dimpled : 1 wrinkled, and when sown would give rise to F_2 plants bearing F_3 seed as follows and in the proportions indicated:

4 plants bearing all wrinkled seed.
6 ,, ,, 3 dimpled to 1 wrinkled.
3 ,, ,, all dimpled.
2 ,, ,, 3 smooth to 1 wrinkled.
1 plant ,, all smooth.

In the trials under review only the dimpled F_2 seed was sown from a cross between Greenfeast (wrinkled) and Blue Prussian (smooth). Results are recorded in Table VI.

Table VI. *Form of F_3 seed arising on F_2 plants of Wrinkled $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ Smooth when the wrinkle-seeded variety contains one factor for dimpling and only dimpled F_2 seed is sown.*

	Number of F_2 plants upon which the types arise		
F_3 seed types	Observed number	Observed ratio	Expected ratio
3 D : 1 W	105	7·2	6
All D	45	3·1	3
3 S : 1 W	16	1·1	2
All S	8	0·6	1

Moreover, in back-crosses of smooth on to the F_2 wrinkle-seeded segregates in which Greenfeast was used the expectation is that on one plant in every four the pods developing as a result of the back-cross would produce smooth seed; similar pods on the other three would contain dimpled seeds. Twelve back-crosses were made resulting in just this proportion, namely, 9 containing dimpled and 3 containing smooth.

It is likely that other wrinkle-seeded varieties will prove to contain only one factor for dimpling. The formula $\mathbf{l_1l_1L_2L_2rr}$ has been suggested by Wellensiek for wrinkled, white-flowered, but the probable effect of such a constitution in Smooth × Wrinkled is not discussed.

III. DIMPLED $\underset{\leftarrow}{\times}$ SMOOTH (Pl. V, fig. 1).

From this cross the following was to be expected:

$$\mathbf{L_1L_1L_2L_2RR} \times \mathbf{l_1l_1l_2l_2RR} = F_1\ \mathbf{L_1l_1L_2l_2RR}\ \text{dimpled.}$$
$$\mathbf{l_1l_1l_2l_2RR} \times \mathbf{L_1L_1L_2L_2RR} = F_1\ \mathbf{L_1l_1L_2l_2RR}\ \text{smooth.}$$

With a dimple-seeded mother plant the **RR** is not observed and the F_1 seed is dimpled. In the reciprocal cross **RR** is observed, dimpling being not yet effective, and F_1 seed is smooth. Field results in Table VII confirm this.

Table VII. *Phenotypes of F_1 seed in Smooth $\overset{\rightarrow}{\times}$ Dimpled.*

Dimpled variety	Smooth × Dimpled	Dimpled $\overset{\leftarrow}{\times}$ Smooth
Harrison's Glory	41 S	81 D + 5 "S"
Day's Early Sunrise	—	6 D
Partridge	5 S	8 D + 2 "S"
Imperial Blue	10 S	15 D + 5 "S"
Pride of the Market	25 S + 3 slightly D	14 D + 6 "S"
Totals ..	81 S + 3 slightly D	124 D + 18 "S"

The F_2 seed borne on an F_1 plant of the constitution $\mathbf{L_1l_1L_2l_2RR}$ will necessarily be dimpled since dimpling is epistatic to smooth. Field results confirm this.

Blue Prussian (S) × Harrison's Glory (D)
$= F_2$ 213 "smooth" + 467 dimpled.

Harrison's Glory (D) × Blue Prussian (S)
$= F_2$ 202 "smooth" + 993 dimpled.

It will be seen that the ratio of total "smooth" to dimpled is 1 : 3·5, but again this ratio is claimed to be entirely accidental as has been discussed already under Table III. It is interesting to note in this connection the following comment by Wellensiek:

"Bateson mentioned a case—called an exceptional case by White—that requires further elucidation. It is a case of indent × smooth (white-blossoming). The F_1 seed was indent, as might be expected. The F_2 seed, however, showed segregation into 339 indent, 119 smooth, and 39 doubtful. The expectation was that all the seeds should be indent.

"Kajanus pronounced the opinion that the segregation was only seemingly so, due to modification. This may be so, but then it is a strange and striking contingence that the numbers are pretty conformable to a (3) : (1) ratio."

It is suggested that Kajanus is correct in assuming the appearance of smooth seeds to be due to modification.

F_3 *seed of Smooth* $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ *Dimpled.*

In F_2 **RR** remains constant and segregation is for $\mathbf{L_1l_1L_2l_2} \times \mathbf{L_1l_1L_2l_2}$. Therefore in only one plant in 16, namely $\mathbf{l_1l_1l_2l_2}$, is **RR** expressed in the F_3 seed, resulting in 15 F_2 plants producing dimpled seed and one producing smooth seed.

Field results were not conclusive. In one case of Harrison's Glory (D) × Blue Prussian (S) the ratio was 1 : 16, but in another, 3 plants bore smooth seed, 3 very slightly dimpled to almost smooth, and 93 bore dimpled seed.

Back-crosses.

These proved to be in accordance with expectation as follows:

Dimpled × (Dimpled × Smooth) 27 seeds all dimpled.
Smooth × (Dimpled × Smooth) 65 seeds all smooth.

IV. WRINKLED $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ DIMPLED (Pl. V, fig. 2).

The expectation in Dimpled × Wrinkled would be:

$$\mathbf{L_1L_1L_2L_2RR} \times \mathbf{L_1L_1L_2L_2rr} = F_1\ \mathbf{L_1L_1L_2L_2Rr}\ \text{dimpled.}$$

The F_1 seed has the constitution **Rr**, but is borne on a plant carrying the factors for dimpling and is therefore dimpled.

The reciprocal cross, Wrinkled × Dimpled, also results in F_1 seed being dimpled. This may best be explained by regarding the cross **rr** × **RR** in which the F_1, **Rr**, should be smooth. But since this **Rr** is borne on a wrinkle-seeded parent, and the wrinkle-seeded parent carries dimpling, then the smooth of **Rr** cannot be expressed and the observed result is dimpling. Field results are recorded in Table VIII.

Table VIII. F_1 *seed arising from Dimpled* $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ *Wrinkled.*

Dimpled variety	Wrinkled × Dimpled F_1 seed	Dimpled × Wrinkled F_1 seed
Harrison's Glory	215 D + 18 "S"	198 D
Dimpled Daisy	59 D	66 D + 5 "S"
Pride of the Market	154 D + 15 "S"	69 D + 111 "S"

Further breeding proved the "smooth" to be genetically dimpled.

These results are in accord with those obtained by Bateson and Kilby from Dimpled × Wrinkled. On the other hand, they found Wrinkled × Dimpled to give F_1 smooth. This is not in accord with the results obtained by the writers who offer the suggestion that Bateson and Kilby used for their cross a wrinkled variety of the constitution $\mathbf{l_1l_1l_2l_2rr}$. In such a case **Rr** would be effective, since dimpling is absent and the F_1 seed would be smooth.

F_2 *of Dimpled* $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ *Wrinkled.*

The F_2 seed of **RR** × **rr** will segregate into **RR**, **Rr**, **rR**, **rr**, and all would be produced on F_1 plants having one or more factors for dimpling. The **RR**, **Rr** and **rR** seeds would be dimpled, and the **rr** wrinkled, thus giving a total ratio of 3 dimpled to 1 wrinkled. Field results gave F_2 seed as follows:

$$\left.\begin{array}{l}\text{578 “Smooth”}\\ \text{2089 Dimpled}\end{array}\right\}\ \text{2667 to 940 wrinkled.}$$

Ratio 2·84 to 1.

The F_2 plants grown from the wrinkled seed should remain constant.

The F_2 dimpled seed on the other hand should give one plant bearing all dimpled seed to two bearing dimpled and wrinkled in the ratio of 3 : 1.

The number of plants grown from the dimpled seed was not sufficient to confirm this. Actual results were 27 plants bearing dimpled and wrinkled seed in the ratio of 3 : 1, to 9 plants bearing only dimpled seed. The proportion should have been 24 : 12.

Check-crosses.

Check-crosses were made, namely, Smooth × (Dimpled × Wrinkled). These should give all F_1 smooth seeds, and the cases were proved by field results.

The F_1 plants of this check-cross should comprise one plant bearing all dimpled seed to one bearing dimpled and wrinkled.

Field results were as follows:

	No. of plants producing	
Varieties crossed	3 D : 1 W	All D
Blue Prussian S × (Greenfeast W × Harrison's Glory D)	8	11
Blue Prussian S × (Harrison's Glory D × Greenfeast W)	8	7
Blue Prussian S × (Great Crop W × Harrison's Glory D)	5	3
Totals...	21	21

SUMMARY.

1. The shape or surface form of garden and field peas may be smooth, wrinkled or dimpled, depending upon the genetic constitution. The appearance of these forms is discussed and some comments made on the manner in which the characters are transmitted and expressed.

2. The genetic formulae of the three forms as suggested by other investigators do not explain satisfactorily the results observed from numerous crossings effected by the writers.

3. The following alternative combinations are offered as forming the constitution of the commercial varieties utilised:

Smooth form $\mathbf{l_1l_1l_2l_2RR}$.
Wrinkled forms $\mathbf{L_1L_1L_2L_2rr}$ and $\mathbf{l_1l_1L_2L_2rr}$.
Dimpled form $\mathbf{L_1L_1L_2L_2RR}$.

4. The direct effect of the various factors is masked by the interaction of one upon the other. Smooth is dominant to wrinkled, dimpled is epistatic to smooth, and wrinkled is epistatic to dimpled.

5. The appearance of the seed in the F_1, F_2 and F_3 generations of reciprocal crosses between smooth, wrinkled and dimpled varieties is described and the resultant observations explained by the application of the suggested formulae.

6. The results from back-crosses and check-crosses confirm the adoption of the alternative combinations.

7. It is supposed that other varieties exist which differ in constitution from those by means of which the present facts regarding the mode of inheritance of seed surface forms have been determined.

ACKNOWLEDGMENT.

The writer's thanks are due to Mr H. Drake who supplied the photographs for the plates.

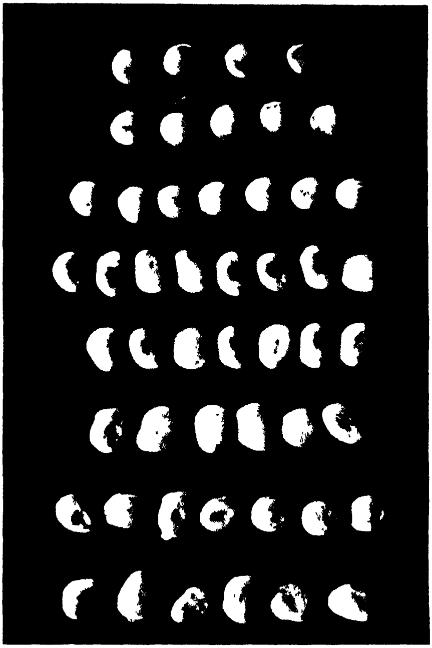

Fig. 1.

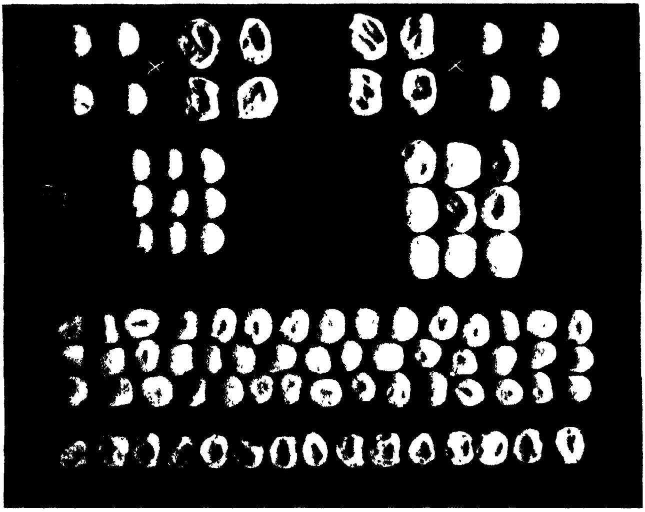

Fig. 2.

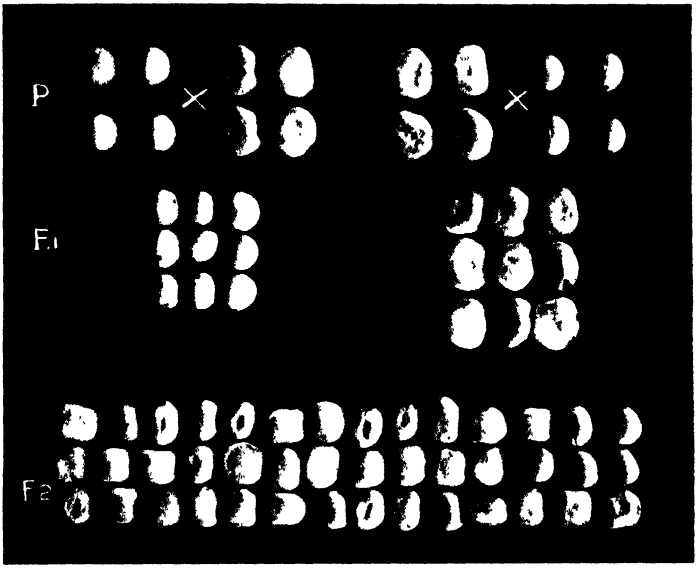

Fig. 1.

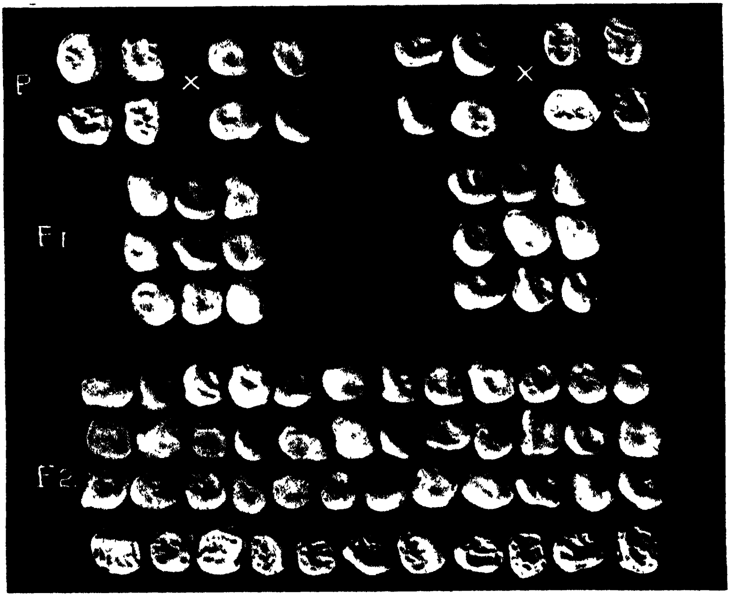

Fig. 2.

EXPLANATION OF PLATES IV AND V.

PLATE IV.

Fig. 1. Demonstrating the origin of "smooth" seeds in dimple-seeded varieties of peas. Each row, the content of a single pod, is arranged in ascending order as the pods grew on the main stem of the mother plant. When, as in this case, both dimpled and "smooth" arise on the one plant, "smooth" seeds are found in the upper or later maturing pods.

Fig. 2. Smooth $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ Wrinkled.

P. Smooth × Wrinkled   Wrinkled × Smooth
| |
F_1. Smooth   Dimpled
F_2. Segregates by seed into 3 dimpled : 1 wrinkled.
F_3 (not illustrated). F_2 wrinkled remains wrinkled.
F_2 dimpled segregates by plant into:
30 bearing F_3 seed in the ratio of 3 dimpled : 1 wrinkled.
15 ,, ,, that is all dimpled.
2 ,, ,, in the ratio of 3 smooth : 1 wrinkled.
1 ,, ,, that is all smooth.

PLATE V.

Fig. 1. Dimpled $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ Smooth.

P. Smooth × Dimpled   Dimpled × Smooth
| |
F_1. Smooth   Dimpled
F_2. All dimpled.
F_3 (not illustrated) 15 plants producing dimpled seed.
1 plant producing smooth seed.

Fig. 2. Wrinkled $\overset{\rightarrow}{\underset{\leftarrow}{\times}}$ Dimpled.

P. Wrinkled × Dimpled   Dimpled × Wrinkled
| |
F_1. Dimpled   Dimpled
F_2. Segregates by seed into 3 dimpled : 1 wrinkled.
F_3 (not illustrated). F_2 wrinkled remains wrinkled.
F_2 dimpled segregates by plant into:
2 bearing F_3 seed in the ratio of 3 dimpled : 1 wrinkled.
1 ,, ,, that is all dimpled.

(*Received October* 17*th*, 1934.)

INVESTIGATIONS ON THE ROOT NODULE BACTERIA OF LEGUMINOUS PLANTS.

XVI. EFFECT OF AIR CONTENT OF THE MEDIUM ON THE FUNCTION OF THE NODULE AND ON THE EXCRETION OF NITROGEN.

By ARTTURI I. VIRTANEN and SYNNÖVE von HAUSEN.

(*Laboratory of the Foundation for Chemical Research,
Helsinki, Finland.*)

The factors which influence the functions of the root nodules of leguminous plants have been extensively investigated during the past decades. Although this research has greatly widened our knowledge of the properties and activities of the nodule bacteria, several important questions, both theoretical and practical, are still unsolved.

It is a well-known fact that the effectiveness of the nodule bacteria varies greatly in different types of soil, and that in such cases it is often impossible to find a satisfactory explanation for these variations. In pursuance of our work on the leguminous plants and root-nodule bacteria we have paid particular attention to the effect of the air content of the medium on the activities of the nodule. It is quite natural that an effective fixation of nitrogen should depend upon an adequate supply of air to the nodules. Besides, it may reasonably be assumed that the strictly aerobic nodule bacteria would be incapable of action under anaerobic conditions. Nobbe and Hiltner (1893) came to the conclusion that in water cultures the function of the nodules of leguminous plants is inhibited and that they do not supply their host with nitrogenous food when all nodules are submerged. According to Thornton (1930), the lack of air supply may inhibit nitrogen fixation by bacteria in the leguminous nodule. In agar cultures he found the most efficient nodules at the surface or at the points where the agar had cracked but no nitrogen fixation where all the nodules were deeply embedded in the agar. Different results were obtained by Wilson, Hopkins and Fred, who found that even the nodules formed within the agar can fix nitrogen (see Fred, Baldwin and McCoy, 1932).

These, and several other investigations cited in literature, show clearly the importance attached to the effect of air on the activities of the nodule. Recent research has disclosed certain important new factors, and a thorough study, with improved technique, of the effect of air on the function of the nodule is therefore needed.

Some years ago we were able to show evidence that in sand cultures considerable quantities of nitrogenous compounds are excreted from the nodules into the sand (Virtanen, 1932; Virtanen, v. Hausen and Karström, 1933). Our experiments were made with sterile cultures of peas, the seed being inoculated with a specific strain of the nodule organism. The nitrogenous compounds found in the sand consisted chiefly of amino acids. This finding showed beyond any doubt that the leguminous plants are able to supply other plants with nitrogenous food, *e.g.* grasses, growing in association with the legumes. Earlier findings to the same effect (Lipman, 1912; Virtanen, 1929; Virtanen and v. Hausen, 1930) were not conclusive as proof for the excretion, since in ordinary pot cultures the action of free-living nitrogen fixers cannot be eliminated[1]. Particularly does this objection apply to Lipman's conflicting findings which can be interpreted otherwise than as a result of an excretion of nitrogenous compounds. The literature of associated growth has been assembled and reviewed by Nicol (1934).

EXPERIMENTAL.

The quantities of nitrogen which are excreted into the sand from inoculated, but otherwise sterile, cultures of peas vary considerably in different experiments. This will be seen from the results of some of our latest experiments, to be presented further on. The experiments were carried out during 1933 with the technique for sterile cultures generally employed in this laboratory (Virtanen, v. Hausen and Karström, 1933). Suction flasks were used as culture vessels. The size of the flasks, and the amount of sand used, were varied in different experiments. The nutrient solution was prepared according to Hiltner[2], but contained no nitrogen. The acidity of the medium was adjusted to *p*H 6·5 by an addition of limestone to the quartz sand. The *p*H could thus be maintained fairly constant during the entire period of growth. In each series of experiments, nitrogen determinations from the plants and sand were made periodically to determine whether a relationship existed between the quantity of nitrogen in the sand and the stage of development of the plant. The roots were carefully removed and the sand was sifted through a metal sieve. The quantity of roots which possibly remained in the sand after this treatment was so small that it could not have any influence on the nitrogen content of the sand. It might be mentioned in this connection that the nitrogen content of the entire root system was invariably smaller than the amount of nitrogen found in the sand.

---

[1] According to several workers, the free-living nitrogen fixers thrive particularly well in the rhizosphere (see Starkey, 1929, 1931).

[2] The composition of this nutrient solution was: $Ca_3(PO_4)_2$ 0·25 g., $CaSO_4.2H_2O$ 0·25 g., $MgSO_4.7H_2O$ 0·394 g., KCl 0·25 g., $FeCl_3$ (5 per cent. solution) 3 drops. Distilled water to 1000 ml.

In Exps. 1 and 2 triplicate nitrogen determinations were made from the sand by the official Kjeldahl method, using 100 gm. sand in each determination. The maximum deviation between the results of individual determinations was 10 per cent. In the later series (Exp. 3) we used the following method for the nitrogen determinations.

After the removal of roots the sand was thoroughly mixed and about thirty samples of 100 gm. were weighed into glass beakers. Six of these samples were then treated by the official Kjeldahl method, using 40 ml. of concentrated sulphuric acid per sample. After cooling, 100 ml. of tap water were added to each flask and the flasks were allowed to stand for 10 min. with occasional shaking. The solution was then filtered with suction, using a glass tube which extended to the bottom of the flasks. At its bottom end the tube was provided with a bulb filled with spun glass. The sand was further washed four times with 75 ml. water and the solution was filtered through spun glass. The combined washings were filtered through asbestos to remove calcium sulphate, and the clear filtrate was concentrated in a Kjeldahl flask until all water had evaporated. The sulphuric acid thus recovered was used for the combustion of another six samples of sand. After combustion the sand was washed in the above manner and the solution from each flask was made up to a volume of 300 ml. 50 ml. of each flask were then measured into two Kjeldahl flasks, made alkaline, and distilled into the same receiver. The final distillation thus represents a composite sample of 400 gm. sand, the total amount of sand analysed being 1200 gm. Control experiments showed that this method gives very satisfactory results (see Appendix).

The amount of nitrogen originally present in the sand was 3·7 mg. per kg. This amount was subtracted from the values found in the experiments (except from the control). An uninoculated culture was run for control in each series.

The results of the sand cultures are summarised in Table I.

The plants of Exps. 1 and 2 were grown in spring, and those of Exp. 3 in autumn, but this variation of season showed no relation to the nitrogen found in the sand. The results of the first three experiments, summarised

Table I. *Sand cultures of "Torstai" peas.*

Exp. 1. 1·3 kg. sand; 1-litre suction flasks; 4 sterile seedlings transferred April 27th, 1934					Exp. 2. 2·7 kg. sand; 2-litre suction flasks; 4 sterile seedlings transferred April 4th, 1934						Exp. 3. 3·4 kg. sand; 3-litre Woulff's bottles; 3 sterile seedlings transferred Sept. 20th, 1934						
No. of exp.	Date of harvest and stage of growth		Dry weight gm.	N* mg.	No. of exp.	Date of harvest and stage of growth		Dry weight gm.	N* mg.		No. of exp.	Date of harvest and stage of growth		Dry weight gm.	N* mg.		
1	Control, June 20th	T†	0·530	24·7	1	Control, June 20th	T	0·580		25·3	1	Control, Oct. 16th	T	1·356	16·2	23·9	Controls; not inoculated
		R	0·431				R	0·452					R	0·633	7·7		
		S		*4·0*			S			*7·2*			S			*10·2*	
2	Before flowering, May 22nd	T	1·470	46·9	2	Before flowering, May 22nd	T	1·582	47·6	73·5	2	Before flowering, Oct. 24th	T	1·353	34·2	50·0	Inoculated with *Rh. leguminosarum*, pea strain HX
		R	1·342				R	1·572	25·9				R	0·796	15·8		
		S		**30·8**			S			**37·8**			S			**66·8**	
3	Start of flowering, June 8th	T	2·937	111·9	3	June 8th	T	2·834	48·0	78·8	3	Full pods, Nov. 16th	T	4·515	81·2	96·1	"
		R	1·031				R	2·142	30·8				R	0·807	14·9		
		S		**33·6**			S			**45·4**			S			**90·6**	
4	Full bloom, June 13th	T	4·160	104·3	4	June 13th	T	4·567	90·6	131·2	4	Full pods, Nov. 16th	T	5·092	121·8	143·4	"
		R	1·561				R	2·131	40·6				R	1·071	21·6		
		S		**42·0**			S			**49·2**			S			**99·7**	
5	Pods formed, June 18th	T	4·938	127·5	5	June 18th	T	5·023	83·5	118·8							"
		R	1·253				R	2·007	35·3								
		S		**39·2**			S			**56·7**							
6	June 21st	T	6·558	153·0	6	June 22nd	T	6·293	115·8	154·0							"
		R	1·207				R	2·333	38·2								
		S		**42·0**			S			**60·5**							
7	Almost ripe, June 27th	T	6·362	187·7													"
		R	2·026														
		S		**44·6**													

Nitrogen determinations made in each case on three 100 gm. samples of sand.

Nitrogen determinations made on 1200 gm. of sand, by the improved method described in the text, pp. 280 and 288.

* Nitrogen in sand is expressed in bold figures, except for the control. The total nitrogen content of the sand in all experiments (except the controls) exceeded the value given in bold figures by the amount of nitrogen given for the control, given in italics opposite "Control S".

† T, tops; R, roots; S, sand.

in Table I, show that nitrogen could be found in appreciable amounts amongst the sand throughout the entire period of growth. The quantity of nitrogen thus found amongst the sand tended to be higher during the later stages of growth; this was especially notable in Exps. 2 and 3. In none of our sterile (inoculated) pea cultures could decomposition of the roots or nodules be observed macroscopically even at the end of the growth period; no microscope sections were, however, made. The excretion of nitrogen thus appears not to be a result of the decay of nodules or of the roots of the maturing plant. This result corroborates fully our previous findings (Virtanen and v. Hausen, 1931). Thornton and Nicol (1934) came to a similar conclusion in the related case of the uptake by grass of nitrogen made available by lucerne.

The above results also show that the amount of nitrogenous compounds found in the sand was the greater, the larger the culture flask. This result cannot be attributable to a better growth of the plants in the larger flasks, since in all experiments the differences in the dry weights of the corresponding plants were insignificant. In Exp. 3 with the largest (3 litre) culture flasks the amounts of nitrogen in the sand were considerably greater than those found in Exp. 2, which again were greater than those found in Exp. 1. In fact, the quantity of excreted nitrogen was in some cases practically equal to that found in the plants. Similar relations between the amounts of nitrogen in the plants and sand have been found by us previously (see Virtanen *et al.*, 1933). Thus, for instance, in these earlier experiments we found 141 mg. nitrogen in two pea plants, against 126 mg. nitrogen in the sand. This has led us to the conclusion that the favourable effect of the larger culture flasks on the rate of excretion must be due to an increased supply of air to the nodules in these flasks.

Our experiments with agar and water cultures of peas lend additional support to this view. Reference has already been made to Nobbe and Hiltner's investigations on the growth of legumes in water cultures. However, as they used ordinary, non-sterile water cultures in which moulds and other micro-organisms might have interfered with the results, it was necessary to repeat these experiments with sterile cultures before anything definite could be stated of the effect of air on the activities of the nodule.

We have previously described some of our experiments which showed that inoculated peas grew well in nitrogen-free water cultures, any measurable excretion of nitrogenous compounds into the solution being, however, not noted. In these earlier experiments no particular attention was paid to the level of the medium in the flasks, or to the extent of sub-

Table II. *Water cultures of "Torstai" peas.*

No. of exp.	Exp. 4. One-litre suction flasks; 4 sterile seedlings in each. All harvested on June 16th, at a late flowering stage (flowers and small pods)		Dry weight gm.	N mg.	
1	Control, not inoculated. Entire root system submerged	T*	0·942	25·3	
		R	0·252		
		M	—	0	
2	Entire root system submerged; ample nodulation	T	1·092	17·1	37·0
		R	1·258	19·9	
		M	—		1·0
3	Medium reached almost to the neck of the root; 10 nodules above the liquid surface	T	1·871	28·1	54·4
		R	1·650	26·3	
		M	—		1·7
4	The flask was only half filled with the medium; a great number of nodules above the liquid surface	T	3·413	71·4	96·2
		R	1·774	24·8	
		M	—		1·8
5	The entire root system was initially submerged; on June 2nd, part of the medium was poured off into an attached flask so that the majority of nodules became exposed to air	T	2·678	55·0	75·2
		R	1·389	20·2	
		M	—		1·1
6	Only a small amount of medium at the bottom of the flask; the majority of the nodules exposed to air	T	4·727	98·1	132·0
		R	2·136	33·9	
		M	—		2·1

No. of exp.	Exp. 5. Two-litre suction flasks; 6 sterile seedlings in each. All harvested on June 13th, at an advanced stage of flowering		Dry weight gm.	N mg.	
1	Control, not inoculated. Entire root system submerged	T	1·689	43·6	
		R	0·412		
		M	—	0	
2	Entire root system submerged; ample nodulation within the liquid	T	2·035	26·5	54·9
		R	2·040	28·4	
		M	—		1·8
3	As in No. 2	T	2·310	27·4	51·2
		R	1·820	23·8	
		M	—		1·8
4	Only a small amount of medium at the bottom of the flask; the majority of the nodules exposed to air	T	6·768	186·7	252·0
		R	3·156	65·3	
		M	—		2·1

* T, tops; R, roots; M, medium.

mersion of the roots. We have therefore now repeated these experiments, with due attention to these points. Results of water cultures are summarised in Table II.

Hiltner's nutrient solution (nitrogen-free) was used. The *p*H of the medium was maintained at 6·5 by the addition of limestone. The sterile seedlings were transferred into all culture flasks on April 27th, 1934, and inoculated with an effective bacterial strain (HX). The level of the medium was varied in different flasks. The original level of medium in the flasks was maintained constant through addition of sterile water from an attached flask.

The above experiments with sterile water cultures of peas, inoculated with the specific nodule bacteria, show clearly that, although an ample formation of nodules takes place in the submerged roots, the activity of the nodules is strongly suppressed, obviously due to a lack of air in the liquid. The nodules have, however, not lost their power of fixing nitrogen, as evidenced by the fact that a powerful fixation sets in if the immersed nodules are exposed to air through a removal of part of the medium. A powerful fixation of nitrogen, and a profuse growth of the pea, take place also when the major part of the nodules is situated above the liquid surface throughout the experiment. In some of our experiments we have led nitrogen gas into the liquid medium during the whole period of growth. In these experiments no nodulation occurred. Oxygen, at least in small quantities, applied to the root system, thus seems to be essential for the formation of nodules.

In water cultures the excretion of nitrogenous compounds is very slight, or practically nil, even in cases when the major part of the nodules is above the water surface. The same is true of water cultures of alder[1], as was also found by Roberg (1934). It may be assumed that for an excretion of nitrogenous compounds to occur, intimate contact between the nodule and the medium is necessary. The "aerial" nodules would thus not be able to give up their excretions, even though the flasks be shaken occasionally (as ours were) so that the nodules are washed by the liquid. On the other hand, it could also be assumed that the powerful excretion of nitrogenous compounds in sand cultures might be attributable to a preliminary wounding of the root surface through the sharp-edged sand particles. This would also explain the negative results obtained in water cultures.

[1] Actually, in water cultures the growth of alder with the aid of its root nodules is in no way materially different from the growth of a legume. If the access of air to the medium is prevented, neither the alder nor the legume will make any growth.

In order to gain some information of this highly important question we have made comparative experiments with agar cultures, in which any mechanical wounding of the root hairs is excluded. In agar cultures the supply of air to the roots can be regulated more easily than in water cultures.

The experimental procedure employed was similar to that followed in the experiments with sand cultures, but to the nutrient medium was added 1 per cent. of agar. After sterilisation the medium solidified into a homogeneous mass. During the growth of the plants, sterile water was added from an attached flask to replace the water which had evaporated. If the addition of water was insufficient, shrinkages appeared along the walls of the flask and a great number of nodules thus became exposed to air. The results of agar cultures are summarised in Table III.

The results of the experiments with agar cultures show that a distinct excretion of nitrogenous compounds takes place when the access of air into the agar is facilitated. The greater the extent of shrinkage and cracking of the agar, *i.e.* the freer the access of air, the more nitrogenous compounds were excreted. The absolute amounts of nitrogen in the agar were, however, smaller than those found in quartz sand cultures. Nodule formation was completely prevented by a layer of paraffin or tri-olein over the agar. In our opinion these results show clearly that the excretion of nitrogenous compounds from the nodules is largely dependent on the air content of the medium. The almost negative results obtained with water cultures would thus be attributable to lack of air. Since the excretion takes place also in agar cultures, it cannot be ascribed to a preliminary wounding of the roots, as might have been assumed to be the case in sand cultures.

The above findings throw light on the question of the variations which are often noted in the effectiveness of the nodule bacteria in different types of soil. It is highly probable that a higher or lower air content of the soil will have a decisive influence on the activities of the nodule. An abundant supply of air manifests its importance particularly in the excretion of nitrogenous compounds from the nodules. This will be clearly seen from our experiments with sand cultures, when the amount of excreted nitrogen in the sand was invariably higher in larger culture flasks than in smaller ones, while in both cases the weight of the plants was almost equal. These results will further provide an explanation for our earlier observation concerning the effect of the ratio non-legumes : legumes on the growth of both species (1930, 1931). When oats were grown in association with inoculated peas in nitrogen-free sand cultures, there

Table III. *Agar cultures of "Torstai" peas.*

No. of exp.	Exp. 6. One-litre suction flasks, containing 750 ml. of agar medium with pH 6·5. The initial amount of nitrogen in the agar was 5·0 mg. per flask. Two sterile seedlings were transferred into each flask on April 20th, and inoculated with HX. All plants harvested on June 28th (full pods)		Dry weight gm.	N mg.	
1	Control, not inoculated	T*	0·392	11·6	
		R	0·217		
		M	—	4·5	
2	Agar was allowed to shrink; abundant nodules, particularly at agar surfaces	T	3·787	95·9	121·8
		R	1·859	25·9	
		M	—		13·7
3	As in No. 2	T	3·741	82·7	102·0
		R	1·390	19·3	
		M	—		15·0
4	Access of air to the roots was prevented by a layer of tri-olein over the agar surface. No nodules	T	0·421	11·5	
		R	0·210		
		M	—	1·2	

No. of exp.	Exp. 7. Experimental conditions as in Exp. 6. One sterile seedling transferred into each flask on April 20th. All plants harvested on June 20th (full pods)		Dry weight gm.	N mg.	
1	Control, not inoculated	T	0·118	7·6	
		R	0·111		
		M	—	4·5	
2	The agar was allowed to shrink slightly; abundant nodulation, particularly at agar surfaces	T	1·442	31·4	45·5
		R	0·499	14·1	
		M	—		12·5
3	Marked shrinkage of the agar; ample nodulation, particularly at agar surfaces	T	1·967	53·8	63·2
		R	1·120	9·4	
		M	—		18·3

* T, tops; R, roots; M, medium.

was evidence that, when the ratio oats : peas exceeded 2 : 1, the growth of both species suffered, obviously from lack of nitrogenous food. The large root system of oats probably reduces the oxygen content of the sand to a level which renders the proper function of the nodules impossible. This results in a lowered rate of nitrogen fixation, and both plants suffer early from lack of nitrogen.

In our opinion the excreted nitrogen compounds represent the amino acids which are directly synthesised in the fixation of nitrogen. This view receives additional support from the fact that the proteoclastic effect of the nodules is very slight.

SUMMARY.

Experiments with sterile cultures of peas, inoculated with their appropriate strain of nodule bacteria, have given the following results:

(1) In quartz sand the rate of excretion of nitrogenous compounds from the nodules was directly proportional to the capacity of the culture flask, *i.e.* to the supply of air to the roots.

(2) In water cultures abundant nodules were formed within the liquid medium. However, when all nodules were submerged, the plants grew poorly and their nitrogen content was low. The growth was the better, the more the nodules were situated above the liquid surface. The submerged nodules were, however, fully capable of fixing nitrogen, since the removal of part of the liquid with the subsequent exposure of the nodules to air resulted in a profuse growth of the plants. Nevertheless, in water cultures the excretion of nitrogenous compounds from the nodules was very slight or nil. The nodule formation was completely inhibited when all air in the medium was replaced by nitrogen gas.

(3) In agar cultures the growth of peas was the better, the greater the extent of shrinkage and cracking of the agar, and the greater the number of nodules on the agar surfaces. A free access of air to the nodules also increased the rate of excretion of nitrogen compounds.

The fact that an excretion took place also in agar cultures showed that the excretion, which we have previously observed in sand cultures, is not attributable to a mechanical wounding of the root surface through sharp-edged sand particles, but that the phenomenon in question actually is due to excretion.

Nitrogenous compounds were found to be excreted from the nodules already at a very early stage when the nodules were still growing. Moreover, the rate of excretion was proportionally higher at the early stages than towards the end of the growth period. This makes it highly im-

probable that these nitrogen compounds, as well as those taken up by the host plant, had originated from a decomposition of the nodule proteins.

APPENDIX.

PROTOCOLS OF NITROGEN DETERMINATIONS ON SAND.

The following experiment was made in 1932 (sowing May 2nd, harvest June 9th). Extracts of protocol:

Control, not inoculated.

Dry weight of 2 plants:	Tops ...	0·512 gm.
	Roots ...	0·504 gm.
Amount of nitrogen in plants	...	14·4 mg.
Weight of sand	... ...	3·40 kg.

Nitrogen determinations were made on three 100 gm. samples of sand. Titrations (ml. of 0·1 *N* H_2SO_4) were: 0·5, 0·5, 0·5, with a blank (reagents only) of 0·3. Hence nitrogen in the sand was 0·28 mg. per 100 gm. Total nitrogen in sand was 9·5 mg.

Experiment I, inoculated.

Dry weight of 2 plants:	Tops ...	2·087 gm.
	Roots ...	0·476 gm.
Nitrogen in plants	... ...	88·9 mg.
Weight of sand	... ...	3·40 kg.

Titrations of three 100 gm. samples of sand (ml. of 0·1 *N* H_2SO_4) were: 2·0, 2·1, 2·0 (mean 2·03), with a blank of 0·5. This gave 2·1 mg. nitrogen per 100 gm. Nitrogen in sand was 71·5 mg. To this was added 8·0 mg. nitrogen recovered from washings of the pot. Total nitrogen in sand was 79·5 mg.

Experiment II, inoculated.

Dry weight of 2 plants:	Tops ...	5·148 gm.
	Roots ...	0·640 gm.
Nitrogen in plants	... ...	153·3 mg.
Weight of sand	... ...	3·20 kg.

Titrations of three 100 gm. samples of sand (ml. of 0·1 *N* H_2SO_4) were: 2·3, 2·3, 2·4 (mean 2·33), with a blank of 0·5. Nitrogen in sand was 81·5 mg.; in washings of the pot, 10·1 mg. Total nitrogen 91·6 mg.

Nitrogen determinations by the improved method.

Control on sand alone. 1·20 kg. treated as described on p. 280, and made up to a volume of 1800 ml. (distillation 600/1800). Titration 3·3 ml. 0·05 N H_2SO_4; blank 1·2 ml. Difference was 2·1 ml., or 5·25 ml. per kg. of sand. Nitrogen in sand was 3·7 mg. per kg.

Recovery of added nitrogen. 63·15 mg. of nitrogen (as $(NH_4)_2SO_4$ solution) was added to 1·20 kg. of the same sand (distillation 600/1800). Titration 32·8 ml. 0·05 N H_2SO_4. This less 3·3 ml. (control) was 29·5 ml., equivalent to 20·65 mg. nitrogen per 400 gm. of sand and to 61·95 mg. per 1·20 kg.

Difference: 1·20 mg. = −2 per cent.
Recovery = 98 per cent.

The figures given in Table I are mean values in the sense of the protocols above.

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(*Received December* 15*th*, 1934.)

INVESTIGATIONS ON THE ROOT NODULE BACTERIA OF LEGUMINOUS PLANTS.

XVII. EFFICIENCY OF DIFFERENT STRAINS OF CLOVER NODULE BACTERIA.

By ARTTURI I. VIRTANEN AND SYNNÖVE VON HAUSEN.

(*Laboratory of the Foundation for Chemical Research,*
Helsinki, Finland.)

In the cultivation of leguminous plants the success of the crop is largely dependent on the efficiency of the nodule organism present in the soil. That different strains of the same organism may differ greatly in their efficiency was recognised by several workers towards the end of the last century (Nobbe, Hiltner and Schmid, 1895; Hiltner, 1900). More recently this question has been the subject of extensive study by Fred and his collaborators (Fred, Baldwin, and McCoy, 1932). It has been found that there are serologically different strains, particularly within the species of soybean and alfalfa nodule bacteria, and that these strains differ also in their efficiency. For a summary of such work, see Fred, Whiting and Hastings (1926).

The present authors have paid particular attention to the efficiency of different strains of clover and pea-nodule bacteria (see Virtanen and v. Hausen, 1932). It was found, for instance, that the relative efficiency of different strains of pea-nodule bacteria is very similar with different varieties of peas. This means that a bacterial strain which aids powerfully the growth of one variety of peas is efficient with other varieties.

Investigations concerning the efficiency of different strains of nodule bacteria have so far been limited to annual plants or, in the case of perennial plants, to observations made during the first season. Since, however, the main crops of perennial legumes are obtained during the second and subsequent seasons, the efficiency of different bacterial strains with these plants must necessarily be followed during several years. The present paper gives an account of our work on the efficiency of ten different strains of red clover nodule bacteria during a period of three years.

Of the bacterial strains used, Nos. I–VIII and No. X were isolated from various parts of Finland. The pH values of the soils from which the bacteria were taken varied from 5·1 to 6·1. No. IX was a Swedish strain, kindly sent to us by Prof. Barthel.

The efficiency of different strains was determined with culture experiments in sand, using unglazed, 12-litre earthen jars. The jars were filled with nitrogen-free quartz sand, the *p*H of which was adjusted to 6·5. According to our previous work, this degree of acidity is optimal for both clover and peas.

Each series of experiments consisted of twenty-one jars. Twenty seeds of red clover were sown in each jar. The seed in the control jar was not inoculated, and that in pairs of the remaining jars was inoculated with one of the ten strains under investigation. The set of jars was thus the following: I *a* and *b* (both inoculated with strain I), II *a* and *b* (inoculated with strain II), and so on. The seedlings were thinned to sixteen plants per jar. The seed used in the experiments was the so-called "Tenhola" clover from the Plant Breeding Station, Tammisto.

All jars were watered daily with a nutrient solution of the following composition:

$MgSO_4$ … … … … …	1·2 gm.
KCl … … … … …	1·5 gm.
KH_2PO_4 … … … … …	1·4 gm.
$FeCl_3$ (5 per cent. solution) … …	1 drop
Water, tap … … … …	20 litres

*p*H 6·5

The nutrient solution being nitrogen free, the growth of the plants depended entirely on the fixation of atmospheric nitrogen by the nodule bacteria. This could be easily seen from the control jars, wherein growth ceased as soon as the supply of nitrogen in the seed was exhausted.

In night-time, and on rainy and cloudy days, the jars were kept in a greenhouse, while in fair weather they were taken outdoors. During the winter the jars were kept in the greenhouse at a temperature of 5–10° C.

The seeds were sown on May 7th, 1931. The first leaves appeared on May 16th, and the first flowers on July 10th. All jars were harvested on August 12th by clipping the plants some 3 cm. above the sand surface.

Soon after the seedling stage, differences began to appear in the rates of growth of the plants, due to the higher or lower efficiency of the bacterial strains used. These differences became more and more marked as time went on. The rate of growth was approximately the same, as judged by the eye, in each parallel pair of jars.

Table I shows the weight and nitrogen content of the crop obtained with the first cut. It will be seen that the differences between the parallel jars are comparatively small. The table shows clearly that the different efficiency of the bacterial strain influences greatly the yield and nitrogen content of the crop even in the first cut of the first season. The most efficient strain used (A VI) produced 85 gm. of dry matter and 2·825 gm. of nitrogen per jar, against 27·5 gm. dry matter and 0·776 gm. nitrogen obtained with the weakest strain (A IV). In other words, the best strain produced three times more dry matter and nearly four times more nitrogen than the weakest strain.

It is particularly interesting to note that the larger crops obtained with the most efficient strains also showed the highest percentages of nitrogen. Thus, for instance, strain A VI produced a crop with 3·32 per cent. of nitrogen in the dry matter, against 2·82 per cent. in the crop obtained with A IV. The lowest nitrogen content, 2·49 per cent. of the dry matter, was noted in the crop produced by A III, a very weak strain. These results show that the differences in the size of crops are attributable to differences in the ability of the bacterial strains to supply their host plant with adequate nitrogenous food.

Table I. *Red clover; first year, first cut (August 12th).*

		16 plants				
Bacterial strain	Jar	Fresh weight gm.	Dry weight gm.	N % of dry matter	N gm.	Ordinal number of average N yield
A I	*a*	415·50	52·96	3·0	1·59	*4*
	b	372·37	51·33	3·01	1·55	
A II	*a*	480·53	62·22	2·98	1·85	*3*
	b	538·50	65·54	2·98	1·95	
A III	*a*	286·5	37·67	2·49	0·94	*8*
	b	293·0	39·68	2·48	0·98	
A IV	*a*	198·0	25·12	2·80	0·70	*10*
	b	223·5	29·95	2·83	0·85	
A V	*a*	321·07	35·54	2·86	1·02	*7*
	b	381·0	46·23	2·83	1·31	
A VI	*a*	514·13	81·55	3·33	2·72	*1*
	b	524·0	88·56	3·31	2·93	
A VII	*a*	336·0	42·87	3·21	1·38	*5*
	b	303·0	38·59	3·23	1·25	
A VIII	*a*	287·5	36·16	2·91	1·05	*9*
	b	234·0	30·22	2·86	0·86	
A IX	*a*	424·0	57·23	3·44	1·97	*2*
	*b**	—	—	—	—	
A X	*a*	295·0	36·25	3·07	1·11	*6*
	b	301·0	42·55	3·05	1·30	
Control (not inoculated)		—	0·58	2·31	0·014	

* Discarded owing to belated flowering.

There seems to be no direct correlation between the efficiency of a strain and the *p*H value of the soil from which it has been isolated. In the present experiments the strains which proved most efficient were isolated from fairly acid soils (*p*H 5·1–5·3). This result may be incidental, but nevertheless it shows that acid soils may contain bacterial strains which under favourable conditions possess a very high efficiency.

The aftermath of the first season was harvested on November 4th, 1931. The combined results of the two harvests are given in Table II. The values for both harvests are given for sixteen plants, as an average for two jars.

Table II. *Red clover; first year, first cut plus aftermath.*

	First cut	Aftermath		Total from two cuts		
Bacterial strain	Dry weight gm.	Dry weight gm.	N %	Dry weight gm.	N gm.	Ordinal number of average N yield
A I	52·2	27·8	3·53	80·0	2·552	*4*
A II	63·9	31·9	3·53	95·8	3·030	*2*
A III	38·7	16·6	3·49	55·3	1·542	*9*
A IV	27·5	16·8	3·43	44·3	1·352	*10*
A V	41·0	19·8	3·30	60·8	1·822	*7*
A VI	85·1	28·7	3·65	113·8	3·873	*1*
A VII	40·7	19·1	3·33	59·8	1·947	*6*
A VIII	33·2	18·6	3·40	51·8	1·591	*8*
A IX	57·2	27·0	3·33	84·2	2·867	*3*
A X	39·4	23·7	3·32	63·1	1·993	*5*

The results in Table II show that the differences between the aftermath crops produced by different strains were less marked than in the first cut. This is, at least partly, due to the fact that in late autumn the rate of assimilation is much lowered and, consequently, differences in the nitrogen supply have less effect upon the size of the crop than during the best growing season.

From the beginning of November to the beginning of April 1932 the jars were wintered in the greenhouse at a temperature of 5–10° C. From observations of the number of plants which perished during the winter it was concluded that those plants which were inoculated with the most efficient bacterial strains and whose growth consequently had been best also showed the greatest winter resistance.

In April 1932 all jars were thinned to six plants per jar in order to secure sufficient room for the roots. Although care was taken that in each jar the best plants should be left, it is quite possible that the thinning might nevertheless have caused some unevenness in the material. In the summer of 1932 three harvests were made from each jar: on May 21st, July 14th and September 30th. The total yields of the three harvests from each jar are given in Table III.

Table III. *Red clover; second year, totals of three harvests.*

Bacterial strain	Jar	Dry weight (6 plants) gm.	N % of dry matter	N gm.	Ordinal number of average N yield
A I	*a*	99·9	3·24	3·24	*4*
	b	95·4	3·03	2·89	
A II	*a*	95·8	3·34	3·20	*2*
	b	163·6	3·08	5·04	
A III	*a*	61·7	2·98	1·84	*9*
	b	70·8	2·97	2·10	
A IV	*a*	65·7	2·79	1·83	*10*
	b	45·1	2·96	1·34	
A V	*a*	92·8	2·97	2·76	*6*
	b	76·5	2·92	2·23	
A VI	*a*	117·9	3·42	4·03	*1*
	b	153·8	3·27	5·03	
A VII	*a*	102·9	2·74	2·82	*5*
	b	70·8	3·10	2·20	
A VIII	*a*	68·9	2·87	1·98	*8*
	b	82·0	2·55	2·09	
A IX	*a*	113·1	3·11	3·52	*3*
	b	84·2	3·12	2·63	
A X	*a*	84·5	3·03	2·56	*7*
	b	70·6	3·10	2·19	

It will be seen from Table III that the second year's results from the parallel jars differed considerably from each other. This is probably to some extent due to uneven thinning, as pointed out above. On the whole, however, the proportions between the results obtained with different bacterial strains are qualitatively similar to those observed in the first year's experiments, but there were great differences between the parallel experiments.

During the following winter, from the beginning of October 1932 to the beginning of April 1933, the jars were again kept in the greenhouse at a temperature of 5–10° C. During this time, one plant perished in each of the jars III *a* and *b*, and VIII *a* and *b*. In the jar VII *a*, all plants perished, while in the remaining jars all plants wintered well.

In the spring of 1933 all jars were thinned so as to contain 5 plants.

In order to ascertain the possible effect of a reinoculation with an efficient strain, one jar of each pair was inoculated with the best strain (A VI). In summer 1933 the jars were harvested three times, on June 5th, July 17th and September 25th. The totals of the three harvests per jar are given in Table IV.

Table IV. *Red clover; third year, totals of three harvests.*

Bacterial strain	Jar	Dry weight (5 plants) gm.	N % of dry matter	N gm.	Ordinal number of average N yield
A I	a*	96·4	3·39	3·27	*4*
	b	92·8	3·28	3·04	
A II	a	110·2	3·22	3·55	*2*
	b*	129·3	3·36	4·34	
A III	a*	66·8	3·14	2·10	*8*
	b	73·7	3·17	2·34	
A IV	a*	59·9	2·97	1·78	*10*
	b	62·4	3·17	1·98	
A V	a*	90·8	3·06	2·78	*5*
	b	81·0	3·25	2·63	
A VI	a	114·1	3·25	3·71	*1*
	b*	147·7	3·24	4·79	
A VII	a†	—	—	—	*7*
	b	69·7	3·35	2·34	
A VIII	a	67·0	2·82	1·89	*9*
	b*	64·8	3·30	2·14	
A IX	a*	112·8	3·14	3·54	*3*
	b	89·2	3·39	3·02	
A X	a*	96·5	3·08	2·97	*6*
	b	76·3	3·07	2·34	

* Reinoculated with A VI.
† All plants perished in winter.

The results in Table IV show that the differences between the yields obtained with the different strains are qualitatively similar to those in the first and second year's harvests. The efficiency of the bacterial strains has thus not substantially changed during the three years' time.

It is also evident that reinoculation during the spring of the third year with an effective strain has not improved the growth of the plants which were originally inoculated with poor strains. It is true that, owing to the unevenness of parallel jars, slight increases of crop could scarcely have been noticed in these experiments, but nevertheless it can be concluded that the reinoculation did not cause any marked improvement in the growth of the plants.

The best and worst strains, and also the fourth best strain (No. 1), maintained their relative positions throughout, on the basis of total nitrogen harvested in each year; the relative positions of the other strains underwent only slight changes.

SUMMARY.

The efficiency of ten different strains of *Rhizobium trifolii* was studied with nitrogen free quartz sand cultures of red clover during three consecutive years.

(1) In the first season, distinct differences were noted in the size and nitrogen contents of the crops produced, after inoculation with ten various bacterial strains. These differences remained clearly discernible throughout the experimental period of 3 years: the individuality of the strains had not materially changed during this time.

(2) Reinoculation with the most efficient strain during the third year did not affect the plants.

(3) The loss of plants in winter was greatest in the jars which were inoculated with weak strains.

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(*Received December 27th*, 1934.)

A STUDY OF THE FACTORS INFLUENCING SIZE OF POTATO TUBERS.

By G. H. BATES, D.Sc.

(*Norfolk Department of Agricultural Education.*)

(With One Text-figure.)

INTRODUCTORY.

THE question of the size of ware potatoes has always been one of importance, especially in localities where soil conditions are poor and a bold sample is difficult to obtain. In cases where growers wish to produce a certain quantity of seed the matter is again one of some moment, and may be of greater economic importance than the production of ware.

Since the advent of the Potato Marketing Board, all classes of growers, upon every type of soil, have become interested in the question of tuber size and the factors which govern it. This is especially the case with those who do not cultivate the best class of soil and who may find it hard to meet the demand of the moment with regard to size.

On the other hand, those who cultivate the most fertile soils, such as silt land bordering the Wash, often receive complaints that their sample of ware is too large and coarse, and not suited to the best branches of the trade.

A vast amount of experimental work in the manuring of potatoes has been carried out in this country, and it has demonstrated on numerous occasions that nitrogen exerts a greater influence upon size of produce than any other manurial ingredient.

In the present work the factors investigated are those of spacing and seed size, and their interaction upon one another.

A number of spacing trials have been carried out in different parts of the Kingdom, but in no case does the influence of spacing upon size of ware produced appear to have been subjected to any statistical examination, neither does the influence of size of seed appear to have been considered in relation to spacing.

Spacing trials carried out upon certain of the comparatively poorer potato soils display large differences between the proportions of ware to seed and to chats as influenced by spacing, but on the rich potato lands the differences are not so great nor is the influence upon total yield so great.

In the case of the latter soils a lower proportion of seed and chats is produced than on others, but spacing greatly influences size of ware. In all previous trials on this class of land, the tubers passing over a certain size of riddle, say 1⅝ in., have been classed as ware and no larger sizes of riddles have been introduced to distinguish the different grades of ware.

That a definite relationship exists between seed size and the percentage of ware in the crop has been proved by Salaman (1) in work carried out in the years 1920–2.

From these trials statistical evidence was obtained that small seed (1–2 oz.) produced a lower total yield than large seed (2–4 oz.), but with a higher proportion of ware potatoes and a higher financial return per acre in consequence. Large seed, on the other hand, produced a higher total yield per acre than small seed, but with a lower proportion of ware. The soil upon which the investigation was carried out was of medium fertility.

In these trials the setts were planted at the same distance apart and the question of spacing did not enter into the investigation. Similar work on the influence of seed size upon produce has been carried out by Wallace and Thompson at Kirton (3) on soil of the same type as that on which the writer's trials were conducted. These were carried out in Norfolk, on the silt land in the King's Lynn district, during the years 1932 and 1933. The varieties employed were Eclipse and King Edward. One trial with each variety was laid down each year on statistical lines.

The spacings employed with Eclipse were 10, 13, 16 and 19 in. The King Edward spacings were 10, 15, 20 and 25 in. in 1932 and 12, 16 and 20 in. in 1933. In all the trials the distance between the rows was 28 in.

The crop was graded over a 1⅝-in. riddle, and all that was retained was reckoned as ware.

In the case of Eclipse there was no significant difference in yield up to 16 in., but there was a significant fall at 19 in.

In the case of King Edward there was no significant difference in yield up to 20 in., but a significant fall after 20 in. (1932). In the second trial (1933) there were no significant differences between 12, 16 and 20 in., the latter spacing being the extreme in this trial.

In all the above cases there appeared to be an increase in size of tuber on the wider spacings.

In 1934 it was decided that a trial should be laid down to estimate the increase in tuber size and at the same time to combine the spacing trial with a size of seed trial. In previous trials the size of the seed had ranged from 1¼ to 2 in. grade, and it was felt that this did not give a standardised medium for experiment.

The first part of this paper is concerned with the above trial, while the second part is an account of a separate investigation carried out at the same time and with the same stock of seed with the object of explaining the mechanism whereby size of seed and spacing influence the size of ware produced.

PART I. SPACING AND SEED SIZE IN RELATION TO YIELD AND SIZE OF PRODUCE.

The trial was laid down at Terrington St Clement near King's Lynn on March 23rd, 1934. The soil is a medium silt and the area covered by the trial was of a uniform and level nature in respect of soil and contour. The whole area received the same cultivation and was uniformly manured with a compound potato fertiliser at the rate of 12 cwt. per acre. The land was ridged, prior to planting, in 28-in. rows, and the soil was in the friable condition typical of silts. The stock employed for the purpose of the trial was King Edward direct from Scotland.

The seed was divided by riddling into three grades as follows:

(1) Small seed $1\frac{1}{4} \times 1\frac{3}{8}$ in., average weight 1·0 oz. approx.
(2) Medium seed $1\frac{3}{8} \times 1\frac{5}{8}$ in., average weight 1·5 oz. approx.
(3) Large seed $1\frac{5}{8} \times 2$ in., average weight 2·5 oz. approx.

As regards the spacing of the setts the distances adopted were 12, 15, 18 and 21 in.

The four spacings were laid out in the form of a Latin Square, *i.e.* sixteen spacing plots in all. Each plot was composed of twelve rows and was subdivided into three smaller plots of four rows each for the three sizes of seed. Thus each seed size was repeated four times with each of the four chosen spacings, so that any one seed size was to be found in sixteen plots, and the whole trial comprising the three different seed sizes of the four different spacings occupied forty-eight plots. The above description is illustrated in the plan. Each of the forty-eight plots was approximately 1/80th acre in area, and the two middle rows of 1/180th acre were lifted and weighed. The end plants in each plot were not included in this area.

In laying down the trial two men furnished with suitably constructed measuring sticks were employed. The distance from centre to centre of adjoining setts was accurately measured in every case. Before the ridges were split, a detailed check was made of the plots and it was found that the spacing had been done with accuracy.

The following observations were made upon the trial during the period of growth from planting to lifting time.

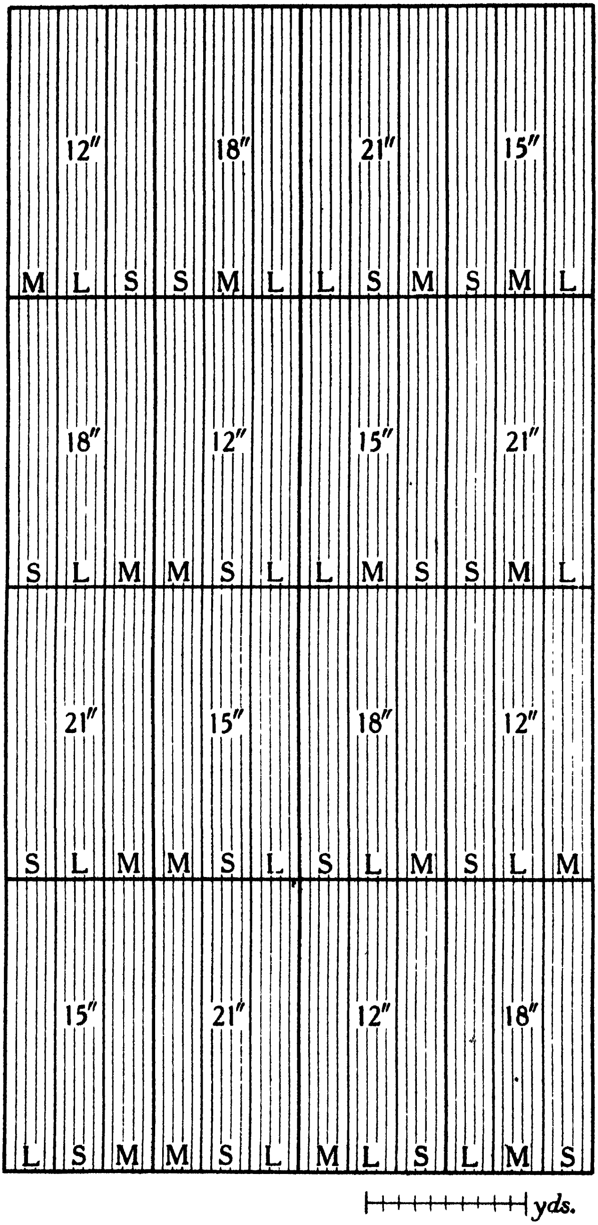

Fig. 1. Plan of combined spacing and size of seed trial.

L = Large seed $1\frac{5}{8} \times 2$ in.
M = Medium seed $1\frac{3}{8} \times 1\frac{5}{8}$ in.
S = Small seed $1\frac{1}{4} \times 1\frac{3}{8}$ in.

The plants were remarkably free from disease, there being no evidence of blight (*Phytophthora infestans*) during the whole season. As regards virus disease a few cases of leaf-drop streak and of leaf-roll were noted amounting to twenty plants infected with the former disease and nineteen with the latter. In no case was the infection severe, and the affected plants were evenly distributed. It bore no apparent relationship to size of seed or spacing.

Apart from the above, the plants were healthy. There was no crowding or shading of one plant by another. A point which will be referred to in greater detail in the next section, but which is worthy of mention here, was the difference in vigour in the growth habit of the plants arising from large seed as compared with those from small seed. Those from large seed had a more vigorous appearance than those from small, and those from medium seed occupied a midway position.

It was found that the apparent vigour was due to the fact that large seed possessed a greater number of shoots than small seed. The average individual shoots of the small seed were as tall and strong in every respect as those of the large seed (see Section II). In habit of growth all plants were upright until the end of the growing period when the widely spaced plants (*i.e.* 18 and 21-in. spacings) became more prostrate and sprawling than the closely planted ones. Size of seed appeared to play no part in this prostrate condition, which was present to the same degree in each seed-size group.

An undetermined amount of second growth occurred after the late summer rains, following a drought period. This was not present to a large extent and appeared to be evenly distributed over the plots, bearing no relation to size of seed or to spacing.

The plots were lifted during the first week in October. The produce of each plot was riddled into four grades, each being weighed. The grades were defined as follows:

(1) Large ware = over 2¼ in.
(2) Ware = 1⅝ × 2¼ in.
(3) Seed = 1¼ × 1⅝ in.
(4) Chats = through 1¼ in.

The above method of separating the ware tubers into two grades gives a better idea of the influence of each respective treatment upon size than could be obtained if the usual practice were followed of defining ware as all tubers passing over say 1⅝ or 1⅜-in. riddle.

A further indication of the differences in size of the large ware was obtained by weighing fifty "large-ware" tubers from each plot and estimating the average weight per tuber.

The results of the trial are set out in Tables I–IX below. Only results which exceed S.E. × 3 are recognised as being significant throughout the whole of this work.

Table I. *Total yields of potatoes (tons per acre).*

	Spacing distances (in.)				
Seed size	12	15	18	21	Mean
Small 1¼ × 1⅜ in.	9·8	10·4	9·3	8·4	9·5
Medium 1⅜ × 1⅝ in.	10·1	10·4	9·9	9·6	10·0
Large 1⅝ × 2 in.	10·9	10·6	11·0	10·0	10·6
Mean	10·2	10·5	10·1	9·3	10·0

S.E. for yield of all seed sizes averaging setting distance = 0·16 ton.
S.E. for yield of all setting distances averaging seed size = 0·24 ton.

Table II. *Yields of total ware over 1⅝ in. (tons per acre).*

	Spacing distances (in.)				
Seed size	12	15	18	21	Mean
Small 1¼ × 1⅜ in.	8·9	9·5	8·5	7·8	8·70
Medium 1⅜ × 1⅝ in.	8·8	9·3	9·2	8·7	9·00
Large 1⅝ × 2 in.	9·4	9·5	9·9	8·9	9·42
Mean	9·02	9·45	9·20	8·45	9·03

S.E. for yield of all seed sizes averaging setting distance = 0·14 ton.
S.E. for yield of all setting distances averaging seed size = 0·24 ton.

Table III. *Yields of large ware over 2¼ in. (tons per acre).*

	Spacing distances (in.)				
Seed size	12	15	18	21	Mean
Small 1¼ × 1⅜ in.	6·3	7·1	6·1	5·6	6·3
Medium 1⅜ × 1⅝ in.	5·4	5·8	6·9	6·2	6·1
Large 1⅝ × 2 in.	4·8	5·9	6·3	5·9	5·7
Mean	5·5	6·3	6·4	5·9	6·0

S.E. for yield of all seed sizes averaging setting distance = 0·18 ton.
S.E. for yield of all setting distances averaging seed size = 0·18 ton.

Table IV. *Average weight per tuber of large ware (lb.).*

	Spacing distances (in.)				
Seed size	12	15	18	21	Mean
Small 1¼ × 1⅜ in.	0·48	0·60	0·60	0·66	0·59
Medium 1⅜ × 1⅝ in.	0·45	0·49	0·50	0·50	0·48
Large 1⅝ × 2 in.	0·39	0·44	0·49	0·45	0·44
Mean	0·44	0·51	0·53	0·53	0·50

S.E. for yield of all seed sizes averaging setting distance = 0·016 lb.
S.E. for yield of all setting distances averaging seed size = 0·021 lb.

Table V. *Large ware expressed as percentage of total ware.*

Seed size	Spacing distances (in.)				Mean
	12	15	18	21	
Small 1¼ × 1⅜ in.	70·0	75·0	72·0	73·0	72·5
Medium 1⅜ × 1⅝ in.	62·0	63·0	74·5	72·0	67·9
Large 1⅝ × 2 in.	51·5	63·0	64·5	66·0	61·2
Mean	61·2	67·0	70·3	70·3	67·2

S.E. for yield of all seed sizes averaging setting distance = 1·4 tons.
S.E. for yield of all setting distances averaging seed size = 0·8 ton.
S.E. interaction between size of seed and spacing of sets = 2·75 tons.

Table VI. *Total ware expressed as percentage of total yield.*

Seed size	Spacing distances (in.)				Mean
	12	15	18	21	
Small 1¼ × 1⅜ in.	90·6	91·5	91·4	93·0	91·6
Medium 1⅜ × 1⅝ in.	87·4	89·4	92·9	90·6	90·1
Large 1⅝ × 2 in.	86·4	89·2	90·0	89·4	88·7
Mean	88·1	90·0	91·4	91·0	90·1

S.E. for yield of all seed sizes averaging setting distance = 0·43 ton.
S.E. for yield of all setting distances averaging seed size = 0·39 ton.

Table VII. *Large ware expressed as percentage of total yield.*

Seed size	Spacing distances (in.)				Mean
	12	15	18	21	
Small 1¼ × 1⅜ in.	63·6	68·1	65·9	67·6	66·3
Medium 1⅜ × 1⅝ in.	53·8	56·1	69·4	65·4	61·2
Large 1⅝ × 2 in.	44·7	56·1	58·3	59·0	54·5
Mean	54·0	60·1	64·5	64·0	60·6

S.E. for yield of all seed sizes averaging setting distance = 1·27 ton.
S.E. for yield of all setting distances averaging seed size = 0·79 ton.

Table VIII. *Yields of seed* 1⅝ × 1¼ *in.* (*tons per acre*).

Seed size	Spacing distances (in.)				Mean
	12	15	18	21	
Small 1¼ × 1⅜ in.	0·77	0·73	0·62	0·49	0·65
Medium 1⅜ × 1⅝ in.	1·13	0·78	0·56	0·73	0·80
Large 1⅝ × 2 in.	1·12	0·94	0·91	0·91	0·97
Mean	1·01	0·82	0·70	0·71	0·81

S.E. for yield of all seed sizes averaging setting distance = 0·035 ton.
S.E. for yield of all setting distances averaging seed size = 0·03 ton.

Table IX. *Yields of chats through 1¼ in.* (*tons per acre*).

Seed size	Spacing distances (in.) 12	15	18	21	Mean
Small 1¼ × 1⅜ in.	0·14	0·15	0·18	0·12	0·15
Medium 1⅜ × 1⅝ in.	0·17	0·31	0·15	0·17	0·20
Large 1⅝ × 2 in.	0·35	0·22	0·19	0·15	0·23
Mean	0·22	0·23	0·17	0·15	0·19

S.E. for yield of all seed sizes averaging setting distance = 0·02 ton.
S.E. for yield of all setting distances averaging seed size = 0·03 ton.

From the foregoing tables the following conclusions may be drawn:

Total yield of potatoes (Table I).

Averaging seed size. Up to and including 18-in. spacing there is no significant difference in yield, but there is a decrease though not statistically significant at 21 in. Taking seed size separately there is a decrease in yield at 21 in. with large seed and with small seed, but not with medium seed. These decreases in yield, however, are not significant.

Averaging spacing. Large seed gives a significantly higher yield than medium or small seed, and medium seed gives a significantly higher yield than small seed.

Yield of total ware (Table II).

Averaging seed size. There is a drop in yield at 21 in., but in this case it is not quite significant.

Averaging spacing. Large seed again gives a heavier yield than medium seed, but there is no difference between small and medium.

Yield of large ware (Table III).

Averaging seed size. In the case of large ware 15 and 18-in. spacing gave higher yields than 12 in. with no significant difference between 15 in. and 18 in.; there was a slight fall at 21 in., but this was not quite significant.

Averaging spacing. There were small increases in yield from small seed over yield from medium and from medium to large seed; but these differences were not statistically significant.

Average weight per tuber of large ware (Table IV).

Averaging seed size. The only significant difference is that 12-in. spacing gives the lowest weight per tuber.

Averaging spacing. Small seed gives large ware tubers of significantly higher average weight than medium or large seed, but there is no significant difference between medium and large seed.

Percentage of large ware to total ware (Table V).

Averaging seed size. The highest proportion of large ware to total ware was given by 18- and 21-in. spacing with no significant difference between them. The 12-in. spacing gave a significantly lower proportion than 15 in.

Averaging spacing. Small seed gave a significantly higher percentage of large ware to total ware than did medium seed, and medium seed gave a significantly higher percentage than large seed.

It should be noted that differences of spacing appear to have no effect upon percentage of large ware to total ware in the case of small seed (Table V), but it is important to note that while this is true, the yield of large ware from small seed is reduced at spacings over 15 in. On the other hand, while size of seed exerts no significant influence upon the absolute yield of large ware (Table III), it does exert a significant influence upon the proportion of large ware to total ware (Table V).

Percentage of total ware to total yield (Table VI).

Averaging seed size. Higher percentage of total ware to total yield was given by 18- and 21-in. spacing than by 15- and 12-in. spacing. The 15-in. spacing gave a higher percentage than 12-in. spacing.

Averaging spacing. Small seed gave a higher percentage than medium seed and medium a higher percentage than large.

Percentage of large ware to total yield (Table VII).

Averaging seed size. There is no difference between 18- and 21-in. spacing, but both gave a higher percentage of large ware to total yield than 15 and 12 in. The 15-in. spacing also gave a higher percentage than 12-in.

Averaging spacing. Small seed gave a higher percentage than medium seed and medium seed a higher percentage than large.

Yield of seed tubers (Table VIII).

Averaging seed size. It is seen that 12-in. spacing gives a significantly higher yield of seed size tubers than 15-in. spacing, and the latter gives a significantly higher yield than 18 in. There is no significant difference, however, between the yield from 18- and 21-in. spacing.

Averaging spacing. Large seed gave a significantly higher yield of seed size tubers than medium seed and medium seed a significantly higher yield than small seed.

Yield of chats (Table IX).

Averaging seed size. The yield of chats in this trial was relatively low and almost negligible. It is found that no significant differences in yield occur in relation to the differing spacings.

Averaging spacing. Large seed gave a significantly higher yield of chats than did small seed, but medium seed was not significantly lower than large seed or significantly higher than small seed.

Conclusions.

Table X below summarises the influence of spacing and seed size (Col. 1) upon total yield (Col. 2), total ware (Col. 3), large ware (Col. 4), percentage of large ware to total ware (Col. 5), seed (Col. 6), chats (Col. 7), and average weight per large ware tuber (Col. 8). In this table are set out the optimum spacings and optimum seed sizes that gave the maximum yields of the various grades of produce etc.

Table X. *Optimum yields.*

(1)	(2) Total yield	(3) Total ware	(4) Large ware	(5) Percentage large ware to total ware	(6) Seed	(7) Chats	(8) Av. wt. per tuber
Effect of spacing	No effect though possibly a decrease at 21 in.	No effect though possibly a decrease at 21 in.	15 or 18 in.	18 or 21 in.	12 in.	No effect	15–21 in.
Effect of seed size	Large	Large	No difference	Small	Large	Large	Small

In cases where practical application may be made of the above Table X, it is necessary to point out that the recommendations with regard to spacing apply only to the variety King Edward. It appears to be very desirable that a similar type of trial to the one under consideration should be carried out with all the popular potato varieties.

PART II. THE RELATION OF SEED SIZE TO NUMBER AND GRADE OF TUBERS PRODUCED.

It has been shown that both spacing and seed size have an influence upon the size of the individual large-ware tubers produced.

In the case of spacing, it is not difficult to understand why a wider distance should increase the size of individual tubers, for competition is lessened and individual plants receive a greater supply of nutrients and moisture. There may also be less tendency to blight (*Phytophthora infestans*) when the shoots and leaves are in a comparatively less crowded condition.

As regards size of seed, the influence upon yield and size of produce is not so readily explained. It is obvious that the problem must be an anatomical or physiological one. In connection with this question, one suggestion offered is that the amount of food material in the parent tuber is an influencing factor (2), in that the larger the seed tuber the greater the initial food supply to the growing plant. It has been shown by Denny (4) that the amputation of the parent tubers at varying stages of the growth of potato plants reduces the yield if amputation takes place before the shoots are 10 in. high. After the plants have reached that height, the presence of the rotting seed tubers appears to be detrimental, in that it exerts a toxic influence upon the growing plant.

While large-seed tubers obviously possess a greater store of food material than do small ones, it is necessary to remember that they also possess more sprouts than small ones and consequently more shoots to utilise this food supply.

It is well known that each seed tuber normally gives rise to several sprouts which reach maturity, but it is not generally realised that the shoot into which a sprout develops must in time become an *individual plant* having an independent existence.

During the time that a sprout is sending out rootlets into the soil and developing leaves above ground level, a constriction is forming at the junction of the sprout with the parent tuber. At any time from within about four to sixteen weeks from planting, depending upon conditions of soil and weather, the constriction closes and completely severs the sprout from the parent tuber. This process is popularly referred to as "weaning".

It occasionally happens that secondary sprouts arise laterally from a primary sprout, and the former break away and carry on a separate existence.

It is obvious from the above facts that the so-called "potato plant" occurring in the field is in reality a group of plants, except in the few instances where the seed possessed only a single sprout. This case is strikingly demonstrated when one member of the group becomes affected with a virus disease such as leaf-roll and the remaining members are apparently healthy and free from symptoms.

Throughout the text this group of plants will be referred to as a "hill". This is a conveniently distinctive American term.

It will be realised that the greater the number of viable sprouts which a seed tuber possesses the greater is the number of individual shoots or true plants which will arise from it.

If tubers are planted at uniform distances in both directions, it is reasonable to assume that a tuber possessing a large number of viable sprouts will give rise to a greater condition of overcrowding than will the tuber with a lesser number of sprouts.

The result of this crowded state would be a comparatively large number of tubers in the produce yielded, these being small in size in comparison with the tubers from parent setts with fewer sprouts. In the latter case the tubers would be fewer and larger.

If such is indeed the fact, then it would follow that large seed would give rise to a greater number of tubers in the produce than would small seed. There are, of course, occasional large tubers with few sprouts or odd small tubers which possess a large number of sprouts. The question of number of sprouts which become active may be a varietal one.

Seed size and number of sprouts.

To test the above theory it was necessary to determine that large tubers actually do possess more sprouts than small ones. A selection was made from King Edward (Scotch) seed and the stock was divided into three groups containing 300 tubers per group as follows:

Size I. Tubers passing over a 1⅝-in. riddle and through a 2-in. riddle, averaging approximately 2½ oz. weight.

Size II. Tubers passing through a 1⅝-in. riddle and over a 1⅜-in. riddle, averaging approximately 1½ oz. weight.

Size III. Tubers passing through a 1⅜-in. riddle and over a 1¼-in. riddle, averaging approximately 1 oz. weight.

Counts of sprouts per tuber were made and the results are set out in Table XI below.

It should be pointed out that the term sprout denotes any "eye" showing viable sprouts. Very frequently an "eye" contains several actual sprouts, but usually only one of these matures under ordinary conditions of storage. Sometimes damage to the apex of a sprout may cause several others to arise from the same "eye". Direct sunlight also produces the same result.

Certain chemical treatment appears to have the same effect as direct sunlight in producing a large number of shoots from one "eye". This was demonstrated by Denny (5) using solutions of ethylene chlorhydrin.

Table XI. *Number of sprouts per tuber in relation to seed size.*

Counts grouped as sprouts per tuber	Percentage of tubers per group 1¼–1⅜ in. 1 oz. seed	Percentage of tubers per group 1⅜–1⅝ in. 1½ oz. seed	Percentage of tubers per group 1⅝–2 in. 2½ oz. seed
0	3	1	—
1	17	8	2
2	25	17	10
3	23	26	6
4	15	31	24
5	11	12	32
6	4	3	14
7	1	1	6
8	—	1	2
9	—	—	2
10	—	—	1
11	—	—	1
Total	100	100	100
Average sprouts per tuber	2·82	3·37	4·55

S.E. of difference between 1 oz. and 1½ oz. seed = 0·15 sprout.
S.E. of difference between 1½ oz. and 2½ oz. seed = 0·16 sprout.

It will be seen from Table XI that the differences between the average number of sprouts per tuber in each group are significant, and that there is an increase in the average number as size of tuber increases.

From each of the above seed-size groups of 300 tubers, 100 tubers were drawn at random, to be used to obtain further information in the field.

The tubers were planted out in a randomised arrangement of plots, and spaced at a distance of 15 in.

Counts were taken at intervals of the number of shoots from each individual "hill", and the results are set out in Tables XII, XIII and XIV below.

The tables show in each of columns (2), (3) and (4), 100 "hills" arising from 100 seed tubers of each respective seed-size class, arranged in groups according to the number of shoots they possess (Col. 1). Thus in Table XII, "hills" possessing three shoots (Col. 1), there are 15 out of 100 in 1 oz. seed (Col. 2), 27 out of 100 in 1½ oz. seed (Col. 3), and 31 out of 100 in 2½ oz. seed (Col. 4).

In Tables XII, XIII and XIV below it will be seen that all the differences are statistically significant.

There was a steady increase in the number of apparent shoots per "hill", but it was found impossible to distinguish true shoots from branches which arose below ground level. This phenomenon made data as to true shoots, at least in Table XIV, inaccurate, but they do illustrate the proportionate number of shoots per "hill" in each of the three seed-size groups.

Table XII. *Number of shoots per "hill" in relation to seed size (counts taken May 25th,* 1934). *(Planted March 23rd,* 1934.)

Shoots per "hill"	Percentage of "hills" per group 1¼–1⅜ in. 1 oz. seed	Percentage of "hills" per group 1⅜–1⅝ in. 1½ oz. seed	Percentage of "hills" per group 1⅝–2 in. 2½ oz. seed
1	43	29	13
2	39	33	29
3	15	27	31
4	2	9	17
5	1	2	6
6	—	—	2
7	—	—	1
8	—	—	1
Total	100	100	100
Average shoots per "hill"	1·79	2·22	2·89

S.E. of difference in average number of shoots per plant with 1 oz. and 1½ oz. seed = 0·13.
S.E. of difference in average number of shoots per plant with 1½ oz. and 2½ oz. seed = 0·17.

Table XIII. *Number of shoots per "hill" in relation to seed size (counts taken June 11th,* 1934).

Shoots per "hill"	Number of "hills" per shoot group 1¼–1⅜ in. 1 oz. seed	Number of "hills" per shoot group 1⅜–1⅝ in. 1½ oz. seed	Number of "hills" per shoot group 1⅝–2 in. 2½ oz. seed
1	21	14	7
2	38	27	32
3	30	24	25
4	6	20	17
5	5	11	14
6	—	2	1
7	—	2	3
8	—	—	1
Total	100	100	100
Average shoots per "hill"	2·36	3·01	3·19

S.E. of difference 1–1½ oz. seed = 0·17.
S.E. of difference 1½–2½ oz. seed = 0·20.

Counts taken later in the season gave still larger numbers of "shoots" with some difference in the proportions. As it was almost impossible to make counts at all at this stage, the figures obtained were discarded.

It will be seen later that counts were made at lifting time when the shoots could be removed intact from the ground and examined. It was then possible to distinguish individual shoots from branches with complete certainty. The figures are set out in the tables below, and it will be seen that they approximate most closely to the results obtained in Table XIII and that the proportions are the same as those in Table XI, XII, XIII and XIV.

Table XIV. *Number of shoots per "hill" in relation to seed size (counts taken July 4th,* 1934).

Shoots per "hill"	Percentage of "hills" per shoot group 1¼–1⅜ in. 1 oz. seed	Percentage of "hills" per shoot group 1⅜–1⅝ in. 1½ oz. seed	Percentage of "hills" per shoot group 1⅝–2 in. 2½ oz. seed
1	16	6	3
2	20	16	1
3	39	19	16
4	24	25	16
5	—	20	30
6	—	7	13
7	—	7	9
8	—	—	10
9	1	—	2
Total	100	100	100
Average shoots per "hill"	2·78	3·86	5·06

S.E. of difference 1–1½ oz. seed = 0·196.
S.E. of difference 1½–2½ oz. seed = 0·23.

Seed size and number of tubers produced.

It was originally intended that each of the hundred "hills" in each of the three seed-size groups should be lifted individually and a record made of the number of shoots per "hill", and the number of ware seed and chat tubers per "hill". It was hoped to lift 100 "hills" per seed-size group, but owing to the heavy work that would have been necessary had this been done it was decided to lift and examine only fifty "hills" per seed-size group. These were selected at random from the 100 "hills", and the results show that the number was sufficient for statistically significant differences to be obtained.

The information obtained is summarised in Table XV below.

Table XV.

	Average shoots per "hill"	Average number of tubers per plant			
		Ware	Seed	Chats	Total
Large seed	3·78	7·70	3·44	2·52	13·66
Medium seed	3·08	7·10	3·18	1·74	12·02
Small seed	2·22	6·58	2·44	1·46	10·48
S.E.	0·24	0·34	0·29	0·27	0·60

From the above table the following deductions may be drawn:

(*a*) The larger the seed the greater the number of sprouts it possesses and the greater the number of shoots it produces, or in other words the greater the number of true plants produced.

(*b*) Large seed produces a greater number of chats than medium and small seed, but there is no difference between the number produced by medium seed and small seed.

(*c*) Large seed produces more seed tubers than small seed, but there is no difference between that produced by medium and small seed.

(*d*) Size of seed has an influence upon number of ware tubers per "hill", large seed producing more tubers than small.

(*e*) Large seed produces a greater number of tubers per "hill" than does small seed.

It is evident that the reason why size of seed does exert an influence upon yield and the grade of produce is mainly in virtue of the fact that the larger the seed the greater the number of true plants ultimately produced. The larger the number of true plants produced the larger will be the total number of tubers, and thus the heavier will be the yield. If spacing is close, the larger the number of tubers the smaller will be their individual size, owing to the greater demand upon the available nutrients in the soil.

GENERAL CONCLUSIONS.

It has been demonstrated that spacing does affect yield of produce and grade of produce by its influence in controlling density of population of "hills" and consequent intensity of competition.

Size of seed influences yield and grade of produce in that it controls the number of true plants in the "hill" and thus controls intensity of competition.

In other words, spacing controls the intensity of competition between the "hills", while size of seed controls the intensity of competition within the "hill".

SUMMARY.

Spacing influences yield in that, beyond certain limits, the decrease in yield is proportionate to the increase in distance between the setts.

The limit to distance between the setts beyond which yield will fall varies with size of seed.

Seed size influences yield, large seed giving higher yields than small seed with the same spacing.

Size of seed influences the size of tubers produced. Small seed gives larger individual tubers than large seed with the same spacing.

Large seed possesses a greater number of sprouts per tuber than medium or small seed. Thus large seed gives rise to a greater number of true plants within the "hill".

The greater the number of true plants in the "hill" the greater will be the number of tubers produced by the "hill" and the smaller will be their individual size.

ACKNOWLEDGMENTS.

My thanks are due to Mr C. W. Cave who carried out the trials, to Messrs E. T. Sykes, M.A., and W. J. West, B.A., who undertook the statistical examination of results, to Dr R. N. Salaman for helpful advice from time to time and for reading and criticising the manuscript, to Mr F. Rayns, M.A., also for reading and criticising the manuscript, and to Dr H. R. Barnell and Mr E. V. Jacks, M.A., B.Sc., for advising in respect to literature.

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(*Received* 30*th January*, 1935.)

VOLUME XXV JULY, 1935 PART 3

AN ELECTRIC OVEN FOR DRYING SAMPLES FROM FIELD PLOTS.

BY F. H. GARNER, M.A., J. GRANTHAM, M.A.
AND H. G. SANDERS, M.A., PH.D.

(*School of Agriculture, Cambridge.*)

(With Plate VI.)

IN field experimentation the effects involved can often only be satisfactorily evaluated on the basis of dry matter yield. The size of plots usually renders the drying of the whole produce impracticable; this necessitates the determination of dry matter content by means of sampling. Since it is essential, on statistical grounds, that each plot should be separately sampled, an experiment may entail the determination of a large number of dry matter percentages. Agricultural produce is generally bulky and subject to fermentation, and therefore apparatus of a larger capacity than that usually found in laboratories is required. In the past much use has been made of uncovered gas heated hot plates, but these are unsatisfactory. Such hot plates do not lend themselves to accurate control of temperature, nor is uniformity of temperature over the plate easy to attain. It is therefore necessary either to run the risk of occasionally burning samples or to dry more slowly than is desirable. Hot plates are very wasteful of heat and the samples are unprotected during drying.

For the above reasons the writers felt the need for some form of large-scale oven. Various methods of heating were considered, but for cleanliness and ease of control electricity was selected. Electric heat being relatively expensive must be used efficiently, and as no suitable lagged oven was on the market a special one was designed in consultation with a firm of electrical heating engineers.

The oven is illustrated below and the following are specifications in brief:

External dimensions.

Height 4 ft. 9 in. (5 in. legs additional), width 5 ft. 4 in., depth 2 ft. 10 in.
Double walls of steel sheets enclosing 1½ in. of insulating material.
Five shelves consisting of removable rod rack grids.

Double doors (with 1½ in. insulation) opening from centre.

Five adjustable ventilators for air inlet in front at base.

One large air outlet at top connected to the ventilating flue of the building and fitted with baffle plate and damper.

Twenty tubular heating elements occupying the whole of the base and controlled by two switches.

Total loading 8·5 kilowatts.

A bimetal adjustable thermostat 3 ft. long, placed centrally near the front of the oven, functioning through the operating coil of a separate contactor switch dealing with the main load.

The heating units are in two groups, which are controlled by separate switches, the odd elements being in one group and the even in the other. There are three settings—high, medium and low—for each switch. When a switch is set at high all elements controlled by it are in parallel: at the medium setting every alternate element of the group is in parallel, the remainder being cut out; when the switch is set at low the elements are arranged as five groups in parallel, each group consisting of two elements in series. Thus it is possible to obtain, by various combinations of the switches, seven different heats, varying from full load down to one-eighth full load. The setting at full load provides for the rapid initial heating of the oven and the load is then reduced according to the temperature which it is required to maintain, the thermostat automatically providing the final control.

The oven has been used principally for drying grass samples. For convenience of working, samples of exactly 100 gm. are weighed out and placed in biscuit tins of the following approximate dimensions: 9 × 8½ × 5 in. Each shelf will take eighteen tins, these being arranged in six rows of three (front, middle and back); thus the total capacity of the oven is ninety tins.

In order to test the uniformity of drying obtained in the oven, a trial was made using moistened sugar beet pulp, which was selected as a material of low variability. The oven was filled to the total capacity of ninety tins; the samples were weighed after twelve hours, eighteen hours and twenty-four hours drying, being replaced after the first two weighings in their original positions in the oven. The thermostat was set to maintain a temperature of 90–95° C., and adequate heating was secured by setting both switches at low. The total consumption during the twenty-four hours was 51·1 units, of which 12·6 units were used in initial heating and in maintaining temperature during weighings. During the operating period, with both switches at low, the average consumption

Plate VI

per hour was 1·886 units, which indicated that the thermostat was cutting out the current for approximately 11 per cent. of the time.

The abbreviated analyses of variance derived from the three weighings were:

	D.F.	$\sqrt{\text{Mean square}}$		
		12 hours	18 hours	24 hours
Shelves	4	1·044	0·324	0·296
Rows	5	1·530	0·316	0·224
Depth	2	0·686	0·000	0·000
Interactions	78	0·555	0·122	0·120
Total	89	0·679	0·154	0·139
General Mean		30·69	30·45	30·41

There was considerable reduction in variation from the twelfth to the eighteenth hour, followed by a smaller reduction from the eighteenth to the twenty-fourth hour. This reduction in variation was closely paralleled by that in mean weight of sample, and it is clear that the greater variation in the early weighings was due to variably incomplete drying. At all weighings shelves gave a significantly greater variance than that due to interactions; this difference persisted to the third weighing, but at that time the extreme variation between shelf means was only from 30·31 to 30·49 gm. In a similar manner rows showed significant differences throughout, but at the final weighing means only varied between 30·35 and 30·49 gm. Depths show no appreciable differences at any time. The above significant differences appeared to be due to a somewhat greater drying on the lower shelves, and in the centre of the oven. The maximum variation was, however, not considered of any practical importance when viewed in the light of the variability of the material for which the oven would normally be used. The "total" root mean square of one dry matter percentage (*i.e.* standard error of determination, with random distribution in the oven) was, after twenty-four hours, only 0·139, and this included such variability as there may have been in the material.

A similar test with forty samples of well-mixed grass gave a corresponding "total" standard error of 0·362 with no significant differences between shelves, rows or depths; this higher figure was undoubtedly due to the greater variability of the material.

It appears justifiable to conclude that the oven works with sufficient precision for all practical purposes, since with materials like grass the error involved in sampling will always be much greater than that due to the determination of dry matter.

(*Received February 1st,* 1935.)

INFLUENCE OF INCUBATION TEMPERATURE ON THE HATCHABILITY OF EGGS, POST-NATAL GROWTH AND SURVIVAL OF TURKEYS.

By ALEXIS L. ROMANOFF, Ph.D.

(Department of Poultry Husbandry, Cornell University, Ithaca, New York.)

(With Four Text-figures.)

The problem of attaining normal development of the avian embryo under artificial conditions is of noteworthy importance from both biological and economic points of view, because the normal pre-natal development of a bird may result in greater probability of normal post-natal development, of resistance to diseases, of increased usefulness and prolongation of life.

As the development of a bird is the result of both heredity and environment, the problem of normal post-natal development may be approached by study either of the influence of the genetic constitution of the embryo, or of the influence of physical or environmental factors of incubation.

While the influence of heredity, particularly the unfavourable effect of inbreeding and the favourable effect of cross-breeding, has been demonstrated, there are to the author's knowledge no data of controlled experiments published on the subject of the influence of physical environment during incubation on the post-natal development of a bird, except that recently published from the author's laboratory on pheasants and quail hatched under various humidity conditions (Romanoff, 1934).

Accordingly, the present study of influence of temperature during the latter part of incubation on the hatchability of turkey eggs and on the early development of hatched poults was undertaken.

Material and methods.

The eggs used in this study were from White Holland turkeys (*Meleagris gallopavo*). For the first three weeks they were incubated in a commercial forced-draft-type incubator (with the revolving paddle) under the conditions recommended by the manufacturer (temperature was 37·5° C. and relative humidity at about 65 per cent.). Then the eggs with well developed and apparently normal embryos, as determined by a powerful egg-lighter, were divided and placed in individual compartments of the electric multiple laboratory incubator (Romanoff, 1932) to complete their development.

The conditions of experimental incubation (from 21 days on) were as follows: Relative humidity was at about 65 per cent.; ventilation was constant with the movement of air just sufficient to maintain a uniform temperature between the upper and lower sides of the egg; and temperature varied in each incubator compartment by 1° C., ranging from 30·5 to 41·5° C. The eggs were turned three times a day, at intervals of about 8 hours, up to 24 days of incubation. Also they were aired and cooled daily throughout the incubation for about 5 min. while they were tested for mortality.

At hatching time the number and the weight of birds, and the original weight of hatched eggs were recorded every 4 hours from the first to the last egg hatched. After about 24 hours from the peak of hatching in each incubator compartment, the apparently normal and healthy poults, in groups not exceeding fifteen individuals, were selected at random and transferred to individual battery brooders. The poults were brooded for 3 weeks under identical conditions and were fed with commercial starting and growing mash for turkeys and supplied with water. Each group of poults was weighed daily, collectively, and weighed weekly, individually.

RESULTS AND DISCUSSION.

Hatchability.

During the 21 days of incubation, that is, prior to the experimental exposure to various temperatures, the mortality of embryos did not exceed 5 per cent. Of this number about 2½ per cent. were dead at the fifth day of incubation—the first well-recognised critical period in the pre-natal development of the bird.

With the exposure to temperatures ranging from 30·5 to 41·5° C. after 21 days of incubation, the second peak of mortality occurred at the 25th day in the groups of extremely high temperature, and at the 28th day in the groups of extremely low temperature. With a medium temperature it occurred, however, at about the 26th day of incubation. These medium or favourable temperatures were 36·5 and 37·5° C., as shown by the highest percentage of eggs hatched from the original number exposed (Table I). Beyond these limits there was a greater slope of decline in hatchability towards high than towards low temperature. Further extremes of selected temperatures (30·5 and 41·5° C.) yielded no hatching.

The variation in the time of hatching, owing to the exposure of the eggs to extremely varied temperatures, was very noticeable (Fig. 1). The groups having peaks of hatching at about 26·5 days were those incubated at the temperatures of 36·5, 37·5 and 38·5° C. Thereafter the time of hatching was progressively delayed toward either high or low temperature. It was delayed for about 1 day at high temperatures (39·5–40·5° C.) and for about 3 days at low temperatures (31·5–32·5° C.).

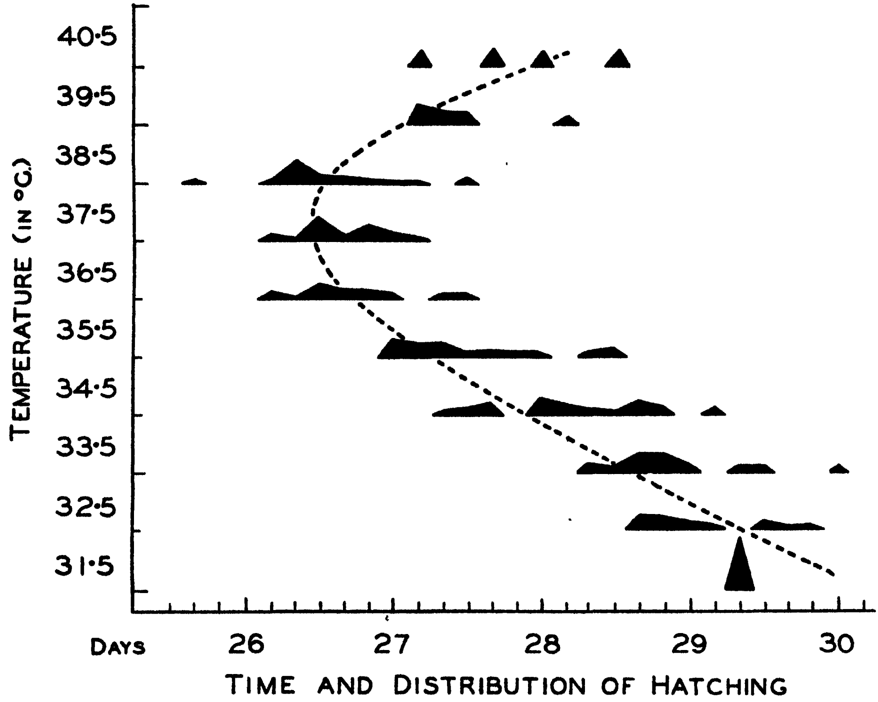

Fig. 1. Influence of temperature on the distribution and the time of hatching of turkey eggs.

The weight of birds at hatching at various temperatures was fairly uniform. There were only slight deviations in the weight from group to group, caused evidently by the variation in the original weight of the eggs. On the other hand differences in the physical condition of the hatched birds were very evident. With a decrease of temperature the number of crippled individuals, those with weak or sprawled legs, progressively increased. Thus under the extremely low temperatures (31·5–32·5° C.) there were practically no birds without this malady. Yet it is interesting to note that high temperatures did not result in any apparent physical abnormality of hatched birds.

Table I. *Influence of temperature on the hatchability of eggs and the physical condition of hatched turkeys.*

Incubation temperature ° C.	Living embryos at 21st day No.	Hatched birds* %	Weight of birds at hatching gm.	Original weight of hatched eggs gm.	Crippled birds at hatching %
30·5	42	0·00	—	—	—
31·5	42	2·38	56·50	(86·00)†	100·0
32·5	56	26·79	57·66	81·78	93·3
33·5	47	44·68	56·05	78·90	33·3
34·5	57	63·16	56·63	80·42	22·2
35·5	50	70·00	57·46	81·75	20·0
36·5	57	78·95	54·77	78·98	2·2
37·5	40	80·00	56·59	77·17	0·0
38·5	57	40·35	56·94	79·73	0·0
39·5	47	17·02	56·00	81·11	0·0
40·5	43	9·30	54·88	83·75	?
41·5	42	0·00	—	—	—

* Based on the number of living embryos at the 21st day of incubation.
† One egg.

Post-natal development.

Relatively good growth was observed with poults hatched at 36·5 and 37·5° C. (Fig. 2, Table II), as compared with the data of Musselıl and Ackerson (1934). The next best group in growth was that hatched at 38·5 and 39·5° C. Still slower growth was observed at the tempera-

Table II. *Influence of temperature on the post-natal survival and growth of turkeys.*

Incubation temperature ° C.	Initial number of birds No.	Survival during 21 days of brooding %	Weight of brooded birds 0 day gm.	7 days gm.	14 days gm.	21 days gm.
31·5	0*	—	—	—	—	—
32·5	12	0·0	53·2	All dead	—	—
33·5	15	0·0	54·0	40·8	All dead	—
34·5	15	33·0	53·1	54·8	98·9	149·1
35·5	15	66·7	58·6	58·8	97·3	154·3
36·5	15	100·0	54·2	78·9	145·7	210·7
37·5	14	100·0	55·6	82·3	133·0	213·5
38·5	12	83·3	51·9	70·0	113·6	178·9
39·5	8	75·0	50·8	60·1	102·7	167·7
40·5	4	0·0	49·8	54·3	All dead	—

Dead prior to brooding.

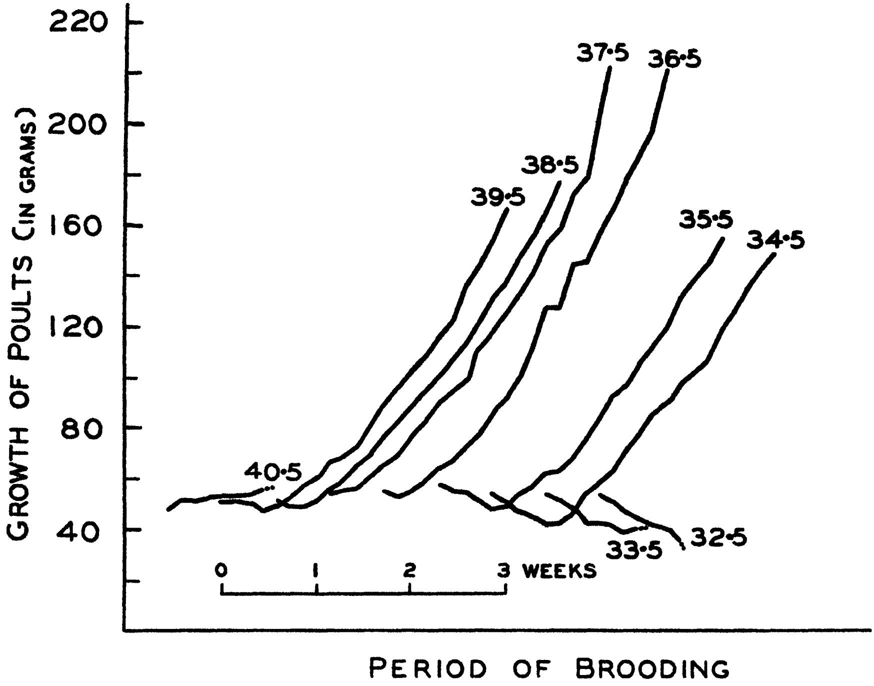

Fig. 2. Influence of incubation temperature on the post-natal growth of turkeys.

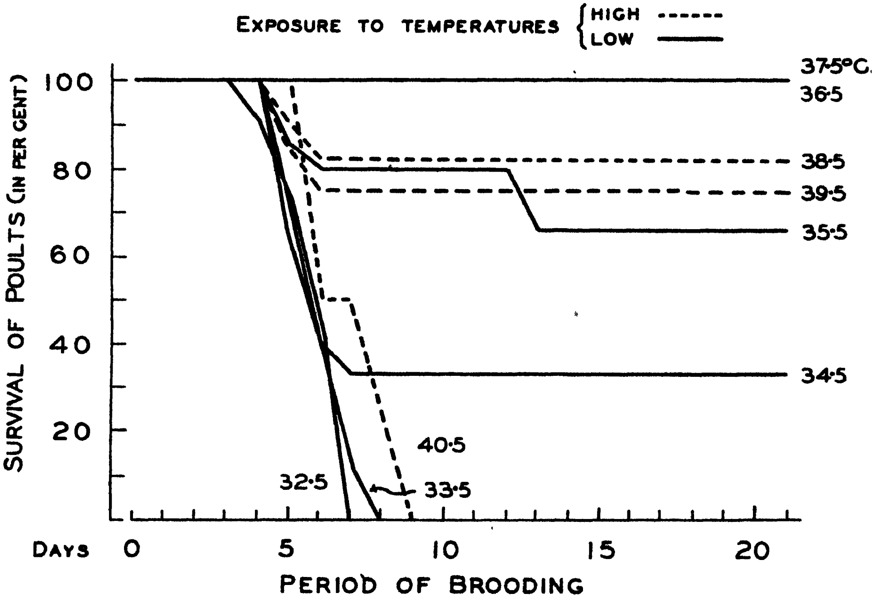

Fig. 3. Influence of incubation temperature on the early post-natal survival of turkeys.

tures of 34·5 and 35·5° C., where the birds for almost 5–6 days refused to eat and consequently for some time did not begin to gain in weight. At 40·5° C. the growth was insignificant, while at 32·5 and 33·5° C. there was no growth whatsoever, because the birds refused to eat or to drink from the beginning, until they were completely emaciated. Consequently, all these birds died at the end of the first week of brooding.

The survival of poults was again the highest—100 per cent.—in the groups which grew fast, that is, hatched at 36·5 and 37·5° C. (Fig. 3). In the rest of the groups the critical period was observed at about the fifth to sixth days of brooding. The total mortality progressively increased in the groups of poults hatched under high or low temperatures. The groups of birds hatched at extreme temperatures (32·5, 33·5 and 40·5° C.) all died within the first week of brooding, presumably through the lack of vitality and consequent inability to take up necessary food not only for growth but for maintenance.

Zones of normal and abnormal development.

From the final analysis of the data there may be seen three distinct and biologically significant zones in the range of temperatures at which the eggs were incubated. Each zone exerted a different effect on the post-natal development of the turkeys.

One zone would include the limits of temperature between 36 and 38° C. suitable for the normal development (Fig. 4). Within these limits of temperature there was the highest hatchability of eggs, the least number of crippled birds at hatching, the best growth and highest survival of poults during the first 3 weeks of brooding. This zone of normal post-natal development it seems is associated with the zone of normal hatchability of eggs.

The second zone on each side of the normal development, towards high (38–40° C.) and low (36–34° C.) temperatures, is the zone of low hatchability, of deficient post-natal growth and high mortality.

At the extreme limits of the range of temperatures used there is a third zone resulting in complete mortality after hatching. The poults hatched at these extreme temperatures neither grew (cf. Fig. 2) nor survived more than 1 week (cf. Fig. 3) although the birds were apparently in good physical health. The limits of such conditions were relatively narrow towards high temperatures (from 40 to 41° C.) but towards low temperatures they were considerably wider (from 34 to 31° C.) and perhaps were of greater significance. While the number of poults in each temperature class is small, the unfavourable results are consistently greater with every increase in the deviation from the optimum incubating temperature for the last week. This suggests that the percentage of hatched birds is not a complete measure of satisfactory

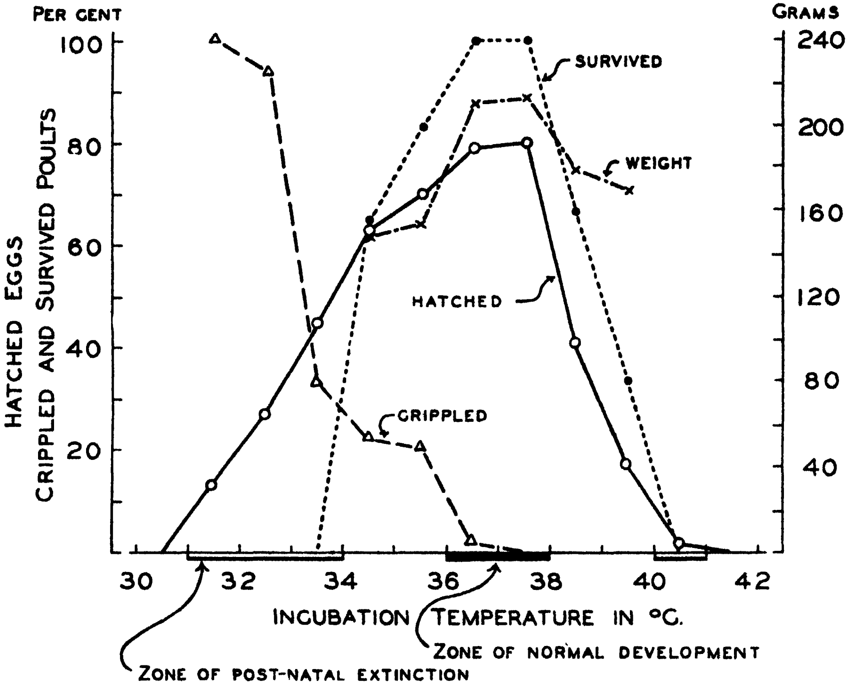

Fig. 4. Influence of incubation temperature on the hatchability of eggs, physical condition of a hatch, and post-natal growth and survival of turkeys.

incubation and that the survival of the hatched birds must be considered. It is not improbable that some of the losses after hatching encountered in practice and in research may be traced directly to unsatisfactory conditions of incubation.

SUMMARY.

The exposure of turkey eggs during the latter part of incubation, to temperatures (uniform around the egg) ranging from 30·5 to 41·5° C., in intervals of one degree, showed that:

(1) The hatchability was best at temperatures of from 36 to 38° C.; then it declined with a greater slope towards high than towards low temperature.

(2) The time of hatching was progressively delayed, from 1 to 3 days, towards high and low temperatures.

(3) The weight of birds at hatching from eggs exposed to various temperatures was fairly uniform.

(4) The number of crippled at hatching progressively increased with decrease of temperature below normal.

(5) The exposure of the eggs to various temperatures produced at least three distinct groups of birds, as the 3-week period of their post-natal development showed.

(*a*) The groups hatched at the temperatures of 36·5 and 37·5° C. were marked by good growth and high viability.

(*b*) The groups hatched at 34·5, 35·5, 38·5 and 39·5° C. were marked by poor growth and high mortality during the first week of brooding.

(*c*) The groups hatched at 31·5, 32·5, 33·5 and 40·5° C. were marked by absence of growth and invariably early death.

The author expresses his indebtedness to Messrs H. J. Grover and O. F. Johndrew for assistance in the care of the laboratory incubators and management of the birds.

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(*Received February* 6*th*, 1935.)

A SOIL MOISTURE METER

DEPENDING ON THE "CAPILLARY PULL" OF THE SOIL. WITH ILLUSTRATIONS OF ITS USE IN FALLOW LAND, GRASS ORCHARD, AND IRRIGATED ORCHARDS.

By W. S. ROGERS.

(*Horticultural Research Station, East Malling, Kent.*)

(With Plate VII and Seven Text-figures.)

INTRODUCTION.

In the course of studies on the root development of fruit plants at East Malling Research Station, an urgent need arose for some quick and easy method for determining the soil moisture content, since soil moisture is one of the important factors which affect root behaviour and plant growth.

The standard method of taking a soil sample and drying it in an oven (1) was found to be very slow and laborious. Moreover, it has serious disadvantages where a series of determinations is required over a long period. Once a boring is made in one position to a depth of 5 ft. or so, another boring cannot be made exactly in that spot.

Accordingly, a survey was made of methods of moisture measurement (see References), and as a result the instrument described later in this paper was constructed. It was thought that a direct-reading moisture meter would have many uses in biological research work. However, it was not until 1931–2, when the author had the privilege of carrying out some months' work at the Dominion Experimental Station, Summerland, which is in the dry belt of British Columbia, that the full value of such an instrument was realised. In the irrigated parts of the world, where the application of water to the soil is under control, a knowledge of the soil moisture conditions, both on the surface and below it, throughout the season is of the greatest importance alike to the grower and to the research worker.

The instrument here described, while it has certain definite limitations, is capable of giving a continuous record of the soil moisture under quite a wide range of natural conditions.

LITERATURE.

It has long been known that the soil exerts a certain "capillary pull" caused by the surface energy of the film of water surrounding its particles and that this pull varies with the moisture content of the soil. In 1908 and 1915, Livingston (24, 25) in his "auto-irrigator" used a porous pot filled with water buried in a pot culture and connected with a reservoir at a stated depth below. The capillary pull of the soil drew water from the porous pot against a constant negative pressure, which thus controlled the amount of water supplied to the soil.

Kornev (23) described in a report on the "Absorbing Power of soils and the principle of automatic self-irrigation of soils" (abstracted in *Soil Sci.* 1924) (23), his use of a porous pot connected to a mercury manometer to measure the "pull" of the soil. The "pull" of the soil on the water in the porous pot caused the mercury to rise until equilibrium was reached. He observed that the "absorbing power" of the soil varies with the "structure, degree of compactness, size of mechanical particles of the soil, and degree of soil moisture". The drier the soil, the greater the pull shown on the manometer.

Haines (14) in 1927 discussed the theoretical side of the relationship between soil and pressure deficiency and threw much new light on this problem. Using a porous pot filled with water and connected to a mercury manometer he obtained moisture: pressure-deficiency curves for a number of uniform media ranging from shot to ignited silt. These showed a sharp rise in pressure deficiency as the percentage of saturation fell from 100 per cent. of the pore space to about 80 per cent., then a gradual rise as the pore spaces emptied from 80 to 20 per cent. of saturation, and a sharp rise below about 20 per cent. of saturation. He mentioned, however (p. 283), that "the main features of the pressure-deficiency curve, namely a distinct bend at each end with a flat intervening portion, become smoothed out as the particle size becomes less uniform, for there is then a smooth and wide graduation in the pore sizes". This is borne out by Kornev's, Heath's and the writer's work.

Heath in 1929 described an apparatus for moisture recording in "A method of water control for sand cultures" (17). A porous cylinder filled with water was buried in the sand of the sand culture. It was connected by a water-filled glass tube to a double-arm mercury manometer. The container was covered to prevent evaporation, and as water was used by the plant the sand became drier and the mercury rose. When water was added the mercury fell. Thirty of these instruments were used in an experiment in which series of containers were kept at two different moisture contents. In each case sufficient water was added daily to the container to cause the mercury to fall to its original level. The author reports that these instruments gave consistent results, and that the readings of the manometer agreed with the amount of water added.

Haines, in a further paper in 1930 (15), raised the important point that the pressure-deficiency curve for rising moisture does not exactly coincide with that for falling moisture, but makes a hysteresis loop. He found that the region of strictly reversible changes was confined to the two ends of the curve.

Keen, reviewing this work in his book *The Physical Properties of the Soil* (21), remarks that failure to recognise this has caused previous work on soil capillarity to be inaccurate or incomplete. The present work bears out that this hysteresis effect does cause a certain error in measurements of actual percentage of soil moisture obtained with a manometer, but in an ordinary soil consisting of particles of mixed sizes this error is comparatively small, as is shown later. Further, it may well be that the capillary pull of the soil, as shown by the manometer, is a more accurate index of the resistance of the soil to the obtaining of water by the plant than is any figure for actual moisture percentage.

Heck (18) reported in 1934 some results in irrigated cane lands of Hawaii with instruments similar to those used by Heath. He suggested that sugar canes gave best growth when the capillary pull was kept (by irrigation) below 25 cm. Hg. On account of the rather limited range of his instruments he considered they could only be used on moist soils.

The instruments described in the present paper have a considerably wider range, however, on account of the refinements that have been introduced.

DESIGN OF THE MOISTURE METER AT EAST MALLING.

The success or failure of this instrument under field conditions depends very largely on the technical details of construction. Many designs and methods of construction were tried by the author, and finally the method of construction detailed in Appendix A was evolved. This has now worked well for more than two years. In brief it consists of a special thick-walled porous pot, with a neck adapted for easy airtight connection to a copper tube which in turn leads to a mercury manometer. The porous pot is filled with water and buried in the soil at the required depth, and the manometer is held in a suitable support. A special device is used in field models to avoid freezing of water in the tube and also to prevent any air which may enter the porous pot dissolved in the soil water from getting into the main tube. Separate filling tubes are filled, to make possible periodical removal of this air and refilling with water without disturbing the rest of the instrument (see Appendix A).

LABORATORY TESTS.

For the laboratory tests carried out at Malling and later at Summerland, B.C., a simple form of instrument as shown in Text-fig. 1 was used. The porous pot *P* and tube are filled with water previously boiled to remove air, and the pot and manometer support are placed in a tin containing a known weight of soil of a known water content. (The manometer support is varnished to prevent absorption of water from the soil.) The whole apparatus can then be weighed on a pan balance and the variations in soil moisture due to subsequent evaporation or addition of water can be correlated with the manometer readings.

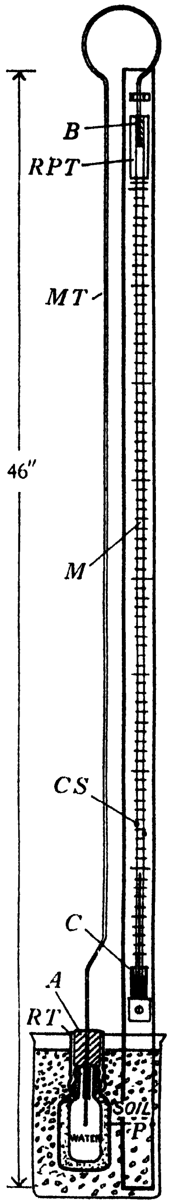


Fig. 1. Moisture meter used for laboratory tests. For full details see Appendix A.

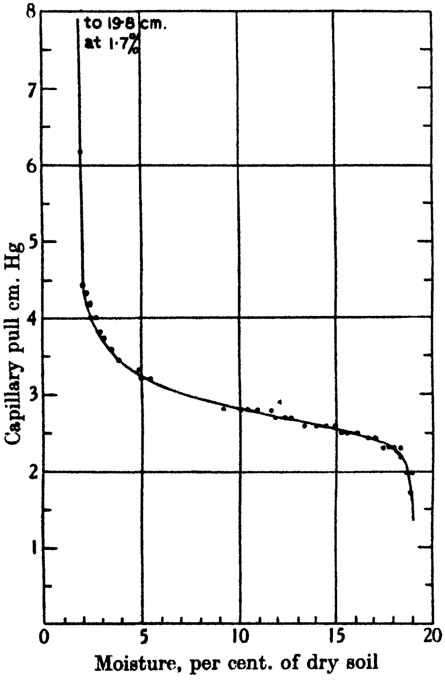


Fig. 2. Moisture: capillary pull curve for sand.

The tins of soil were allowed to dry naturally in the laboratory. The rate of drying was about 0·5–1·0 per cent. moisture per day. Normally readings were taken twice daily (at 9 A.M. and 5 P.M.). Text-fig. 2 shows the curve for sand (drying). This agrees with Haines' curve (15), having a sharp rise at each end and a flatter portion in the middle. The rise over the range of 18 to 4 per cent. moisture is gradual but quite readable. Curves for a heavy clay (Wisboro Green, Sussex) and a fine sandy loam (Summerland, B.C.) are given in Text-fig. 3. Readings for a number of successive dryings and rewettings are given, and the bottom points reached after each successive addition of water are indicated by the Roman numerals. Part of the curve for sand is also shown drawn to the same scale.

The great influence of soil particle size on the capillary pull for a given moisture content is evident. In the clay, the rise of the curve at moistures below 25 per cent. is great and very sharp, and passed beyond the range of the instrument at a moisture content of 21 per cent., which is wetter than often exists under field conditions. The curve for silty loam shows the beginning of a sharp rise at about 15 per cent. moisture, while that for sand (Text-fig. 2) does not rise sharply till 3 per cent. is reached.

The readings for successive wettings of the Summerland loam shown in Text-fig. 3 illustrate the hysteresis observed by Haines (15). When water was added after the mercury had reached 30 cm. in curve I, the mercury fell to 14 cm. (at II) at a lower moisture content that was indicated by a height of 14 cm. on the "drying" curve I; similarly further additions of water gave points III and IV, below curve I. Subsequent curves kept within these extremes. The same effect can be seen in the graph for clay. This gives some idea of the degree of variation likely to be obtained.

Rapid temperature changes will cause variations in the manometer readings through expansion and contraction of the water in the instrument. The daily temperature rise and fall is responsible for the small irregularities in the curves in Text-fig. 3. The laboratory temperature varied between 16 and 22° C. To a certain extent this temperature effect is self-compensating, for as the water expands, tending to let the mercury fall, water will pass out of the porous pot till equilibrium is reached again. A moisture meter in a tin of Summerland loam sealed over maintained a pull of 5·5 cm. over a period of eight days at temperatures varying slowly from 11 to 17·5° C.

The laboratory work showed that for lasting results a special close-grained thick-walled porous pot is essential. In ordinary thin-walled porous pots the film of water filling the pore spaces is liable to break when the pressure deficiency rises above about 35 cm. Hg, so allowing air to pass into the pot, and eventually causing loss of all the water. The pots used in the above experiments were actually made by the writer assisted by a maker of handmade pottery at Summerland, B.C. A number of forms were tested, and the final form used is of considerably greater thickness and density, and is made by a London firm of pottery manufacturers, to the writer's specification. This pot will stand a negative pressure of more than 60 cm. without breakage of the water film. The pressure deficiency that it is possible to measure by this method sets a limit to the range of moisture contents covered. This is approximately as follows: in coarse sand, from saturation to about 1·5 per cent. moisture (on dry weight); in medium loam, from saturation to about 8 per cent.; in heavy clay, from saturation to about 21 per cent.

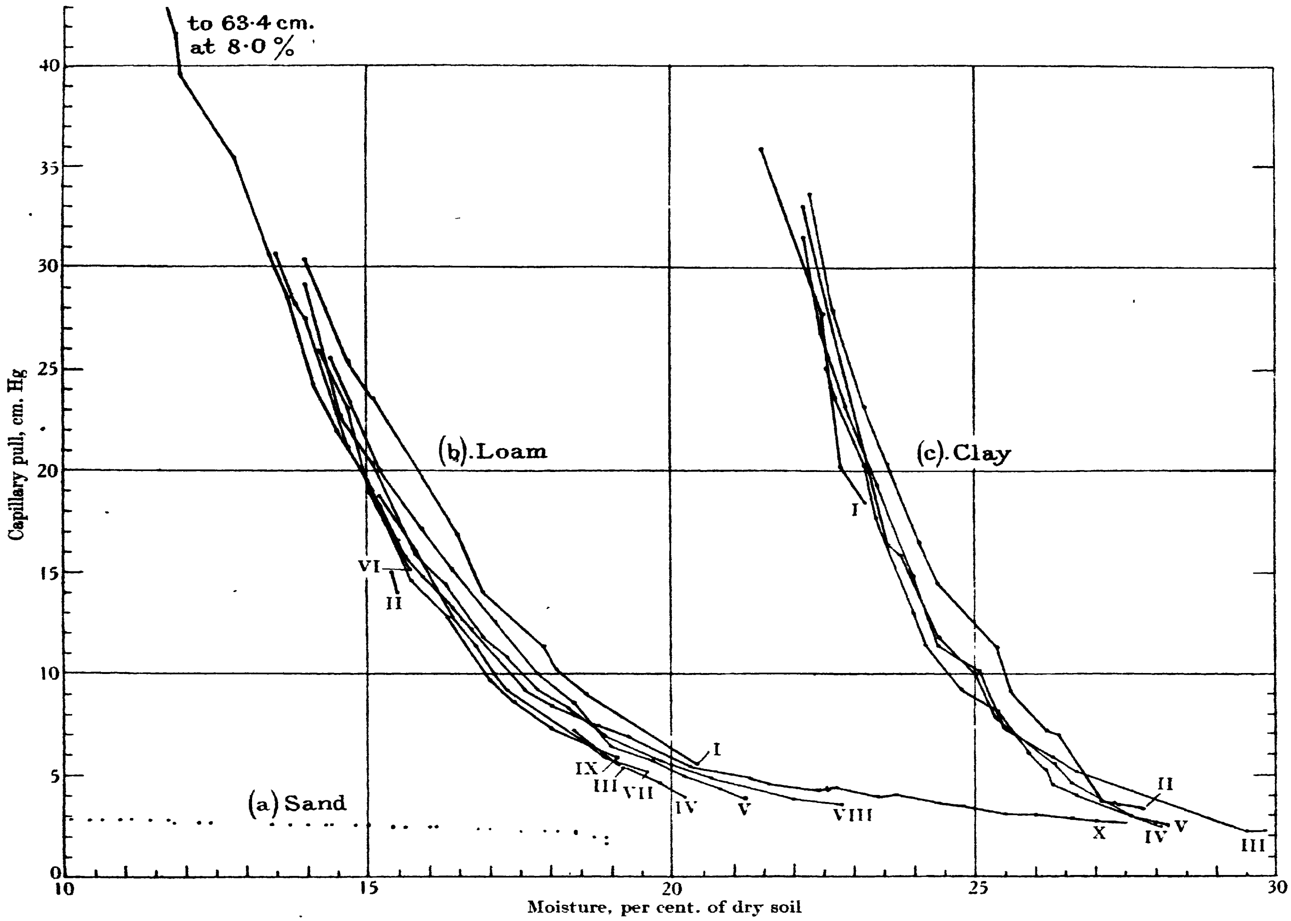
If the capillary pull rises to more than about 60 cm. Hg, the mercury remains at approximately this height, and the water is very gradually drawn from the porous pot, becoming replaced by air. If all the water is drawn out the instrument ceases to function, and the mercury quickly drops to zero. The instrument can be set going again by refilling the pot through the filling tubes (as described in Appendix A). A better plan, however, is to forestall a possible breakdown by refilling the pot at intervals of about a month whenever the capillary pull is very high. After a refill a period of some 24 hours must elapse to allow the instrument to reach a state of equilibrium before readings are taken again.

RESULTS IN THE FIELD.

In the field type of moisture meter the manometer is housed in a sloping wooden casing with a hinged lid, as shown in Pl. VII, which is a double instrument having two pots at different depths—each connected to its own manometer. The slope magnifies the manometer measurements and makes reading easier.

A series of daily readings of the instrument installed at Malling in May 1930, with the rainfall, is shown in Text-fig. 4. The pot of this instrument reached from 6 to 12 in. below the surface of the soil—a light loam, kept free from vegetation. The diagram shows very clearly how the instrument responded to the rainfall. In dry periods the mercury gradually rose, and when rain fell in sufficient quantity to reach the subsoil the mercury fell.

Evaporation caused a fairly brisk drying out of the soil at this depth, but there was sufficient rainfall to keep the capillary pull down to less than 28 cm. Hg, while with the heavy rains at the beginning of August (1·4 in.) the soil at this depth became almost saturated for a short while.


to 63·4 cm.
at 8·0 %
Capillary pull, cm. Hg
0
5
10
15
20
25
30
35
40
(a) Sand
(b).Loam
(c).Clay
VI
II
IX
III
VII
IV
V
I
VIII
X
Moisture, per cent. of dry soil
10
15
20
25
30

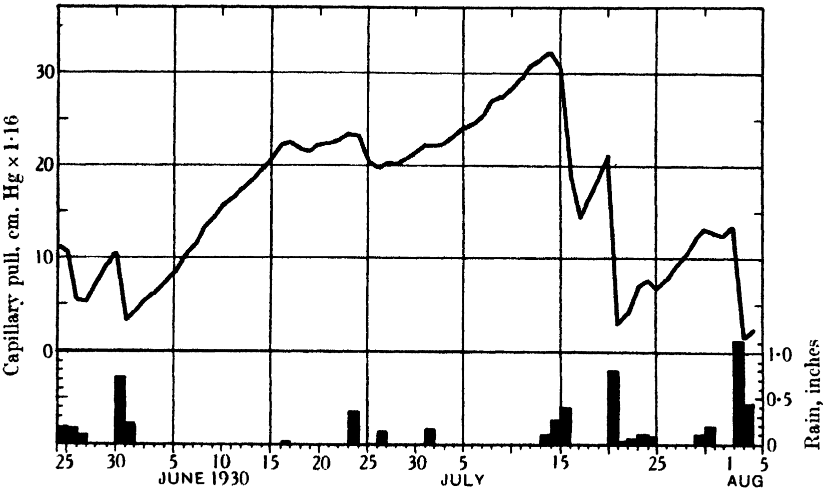


Fig. 4. Correlation between rainfall and capillary pull on a plot of fallow land at East Malling.

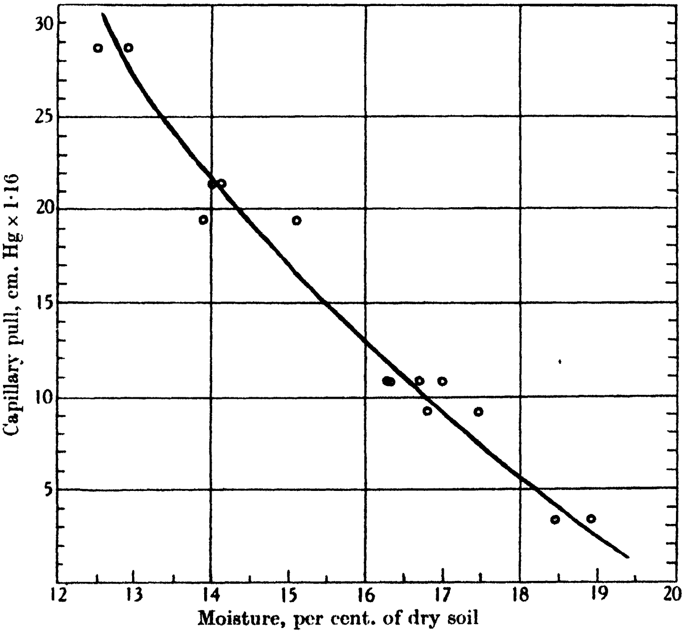


Fig. 5. Relationship between capillary pull and soil moisture on the same plot as in Fig. 4.

The relationship between the mercury rise and actual soil moisture can be seen in Text-fig. 5. Each pair of circles shows the moisture percentages (on dry weight of sample) of borings taken in duplicate at the same time, and at the same depth as the porous pot, within a metre of the pot. The difference between them is due to natural soil variation and to unavoidable errors in the moisture determination. Text-fig. 5 also bears out the point, noted by Conrad and Veihmeyer (4) and many other workers, that no single moisture content figure can be taken to represent with great accuracy any large area, for very large variations occur in quite small distances.

Reduction of these figures to a basis of "relative wetness" by dividing the moisture content by the moisture equivalent of the sample, as suggested by Conrad and Veihmeyer, would probably have reduced the variation. Unfortunately no apparatus for determining moisture equivalents was available. However, even the "relative wetness" was found by Conrad and Veihmeyer to give a coefficient of variability of 9·4 per cent. between six borings made in a circle of 1 ft. diameter (4). Any figure for soil moisture must therefore be regarded as only an approximation to actual conditions.

Thus it would appear from Text-fig. 5 that the manometer reading has varied reasonably consistently with the percentage of moisture in similar soil at the same depth.

SOIL MOISTURE IN FALLOW LAND AND GRASS ORCHARD LAND.

A fuller and more interesting illustration of the action of the moisture meters is shown in Text-fig. 6. This shows the capillary pull of the soil at two depths in clean cultivated land and also in grass orchard land, with the rainfall, for a year at East Malling. At the beginning of the graph the pull at 9 and 30 in. in fallow ground only is shown. It can be seen how the soil at 9 in. (thick dotted line) dries out much more rapidly than the soil at 30 in. (thick continuous line). Also when rain falls the soil at 9 in. approaches saturation more rapidly than that at 30 in., as would be expected. Since this plot was kept free from plants, the decrease of moisture indicated by the rising line is due to evaporation alone. It can be seen that by the end of August 1933, the soil at 9 in. was very dry, since the small amounts of rain in August were quite insufficient to make up for evaporation. The soil at 30 in. in fallow land, although drying slightly, was still very moist, however. At the beginning of September a heavy rainfall almost saturated the soil at 9 in., and percolated more gradually to 30 in. After this the soil kept relatively moist until the

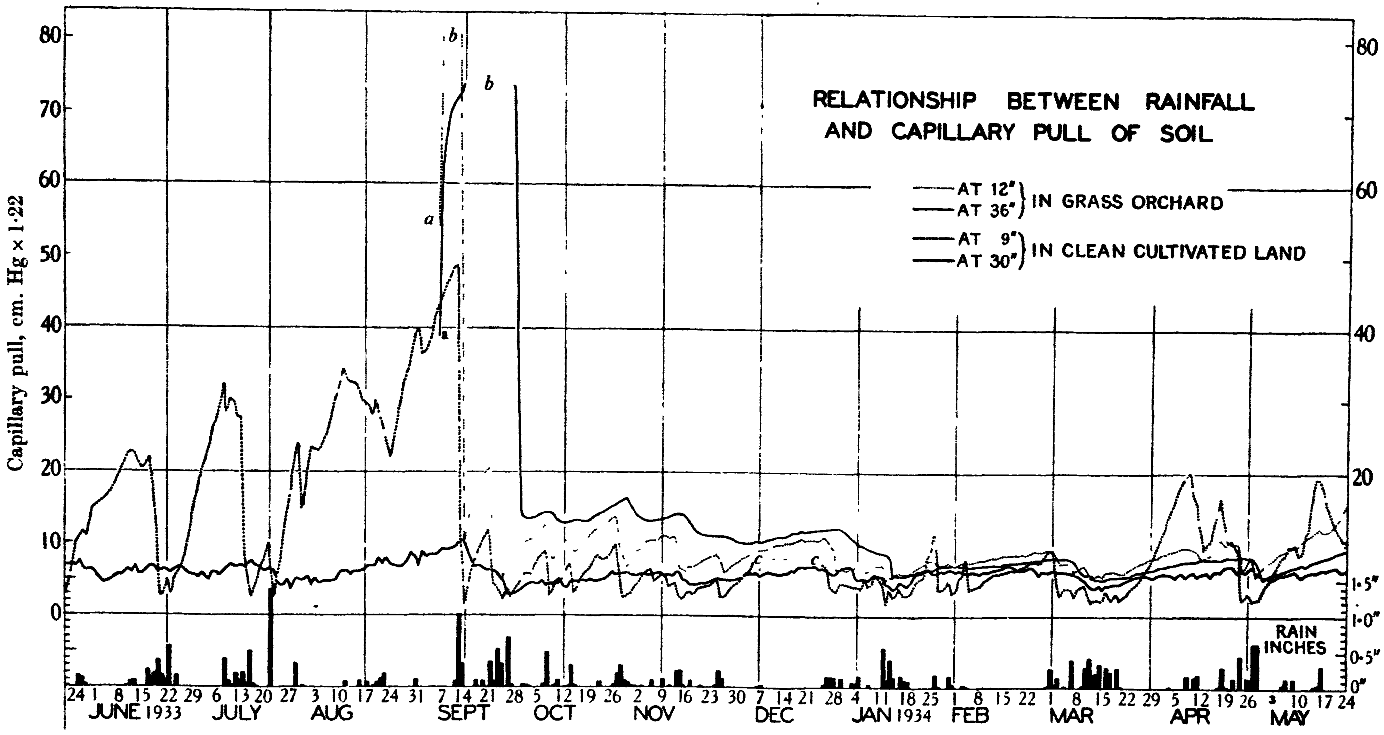


Fig. 6. Rainfall and capillary pull at two depths in fallow land and grass orchard at East Malling.

following March, when the drying winds began to desiccate the soil down to 9 in.

An interesting contrast is provided by the two instruments installed below 19-year-old apple trees in a nearby grass orchard. At the time the instruments were installed (point *a*, Text-fig. 6) the soil was already remarkably dry, in fact the leaves of the trees were showing drought symptoms. The loam at 1 ft. had a moisture content of 6·9 per cent. and the sandy soil at 3 ft. had a moisture content of 4·0 per cent. (mean of two samples). The mercury in both the 1 and 3 ft. meters quickly rose to beyond the top of the glass tube, as shown at *b*. Extra height was hastily provided by bending up the copper tubes to give a total effective height of 88 cm. Hg. This was sufficient to prevent the mercury from being pulled over into the porous pot. On September 14, just a day after the heavy rain had reached 9 in. on clean ground, the water reached 1 ft. in grass, bringing the capillary pull down to 10 cm. But so dry was the orchard soil, and so great the demand from tree and grass roots for water, that it was not until 16 days later, when 3·56 in. of rain had fallen since the drought period, that the water reached the 3 ft. meter. This bears out the contention of Veihmeyer and Hendrickson (32) that if moisture is applied to the surface of the soil it does not become distributed evenly through the whole soil mass, but wets it to "a depth depending on the water-holding capacity of the soil and its initial moisture content", and that further movement by capillary action is very slow.

It will be noted that in January the capillary pull at all depths on both plots is about 7 cm., and it would appear that this is probably about normal when the soil is saturated to its "field capacity". In March the soil at 9 in. on clean ground dries out much more rapidly than that at 12 in. in the grass orchard, probably due to the greater exposure to wind of the former. In other months both meters in the grass orchard usually show a greater pull than the corresponding meters in clean ground. This is undoubtedly on account of the root action of the grass and the trees. It can be seen that the drying out of the soil in the grass orchard took place almost equally at 12 and 36 in., showing that it was the plant roots that were using most of the water. Excavations at East Malling have shown that apple roots penetrate to well over 3 ft. in this soil (30).

SEASONAL CHANGES OF MOISTURE.

The general conformation of the graph shows well the seasonal rise and fall of moisture. The soil reservoir is well filled after the winter rains, and becomes more or less depleted during the summer—to be refilled in winter again.

The limitations of the simpler types of instrument are visible in this graph. The two instruments in the grass orchard had the glycerine anti-freezing device mentioned above and gave consistent results throughout. The instrument at 9 in. in clean cultivated land had water in the manometer tube, and this froze in the period of December marked *C–C*, giving a zigzag and entirely erroneous reading (without bursting the tube, however, on this occasion). The instrument at 30 in. in clean cultivated land had air in the manometer tube, which, while it obviated freezing, was responsible for the very wavy nature of the line, seen in the graph, due to temperature fluctuations. This variation is seen to be several centimetres at times, and cannot be considered satisfactory when compared with the smooth line of the other deep instrument containing the glycerine.

THE MOISTURE METER IN AN IRRIGATED ORCHARD.

The writer has published elsewhere (29) the results of observations in an irrigated apple orchard near a root observation trench in Summerland, B.C. Porous pot meters were placed at depths of 12 and 36 in. at a distance of 18 in. from irrigation furrows, which were 6 ft. apart. The soil dried out gradually from May to September at both depths. Irrigations of 2·5 in. of water affected the 36-in. meter, but rainfalls of 0·5 in. failed to do so. It was observed that the irrigation water penetrated more rapidly into moist than into dry soil. Thus when the soil at 1 ft. showed a capillary pull of about 17 cm., an irrigation of 5·5 in. of water reached both meters within 24 hours of commencement of irrigation, but at the end of August when the capillary pull was 50 cm. an application of 5 in. of water had no effect on the meters until 48 hours after irrigation. Further, the fall in capillary pull was much slower than it had been earlier in the season when the soils were moister throughout.

FURTHER DEVELOPMENTS.

The application of the principle of this instrument has a number of interesting possibilities, beyond those already mentioned. By attaching the porous pot to a recording vacuum gauge, an automatic record of the soil moisture changes has been obtained at Malling for the last two years. Similarly by using a vacuum-operated electric switch, an alarm can be operated or an irrigation system brought into action automatically when the soil reaches a predetermined state of dryness.

Further investigations to increase the accuracy and scope of this instrument are in progress.

SUMMARY.

1. A soil moisture meter which gives direct and continuous measurement of the soil moisture content is described. The instrument consists of a special porous pot filled with water, connected by a tube to a mercury manometer. The pot is buried in the soil, whose capillary pull causes the mercury to rise. The height to which the mercury rises depends on the amount of moisture in the soil, and also on the size of soil particles and the degree of compactness of the soil. (The last two factors remain constant for an instrument in one position.)

2. To read actual moisture percentage each instrument has to be calibrated for the soil in which it is placed. Once this is done, all sampling and weighing is eliminated.

3. The range of the instrument in its present form runs from saturation to about 1·5 per cent. moisture (calculated on dry weight) in sand, to about 8 per cent. in light loam, and to about 21 per cent. in heavy clay.

4. Within this range increases and decreases of soil moisture are recorded rapidly and consistently.

5. This type of moisture meter is prevented from giving absolutely precise readings of the soil moisture content owing to the fact that the moisture : pressure-deficiency curve tends to form a hysteresis loop, *i.e.* does not follow exactly the same course for rising moisture as for falling moisture. The degree of accuracy appears to be sufficient for many purposes, however, for the variability is usually within 10 per cent.

6. Special devices are used to prevent freezing and enable the instrument to give a record over long periods without attention in the field.

7. Examples of the working of this moisture meter in the laboratory and in the field are given. Instruments placed at different depths in clean cultivated land and in grass orchards have shown the contrast between loss of water by evaporation, which hardly affects the soil moisture at 30 in. and loss by root absorption which draws on the deeper layers as well as the surface layers of the soil.

8. In an irrigated orchard the meters have satisfactorily shown the penetration of irrigation water and the drying out of the soil at various depths.

ACKNOWLEDGMENTS.

The author acknowledges with gratitude assistance received from members of the staffs of East Malling Research Station and the Research Departments at Summerland, B.C. He wishes particularly to thank Mr W. A. Roach for help in the early stages of the work.

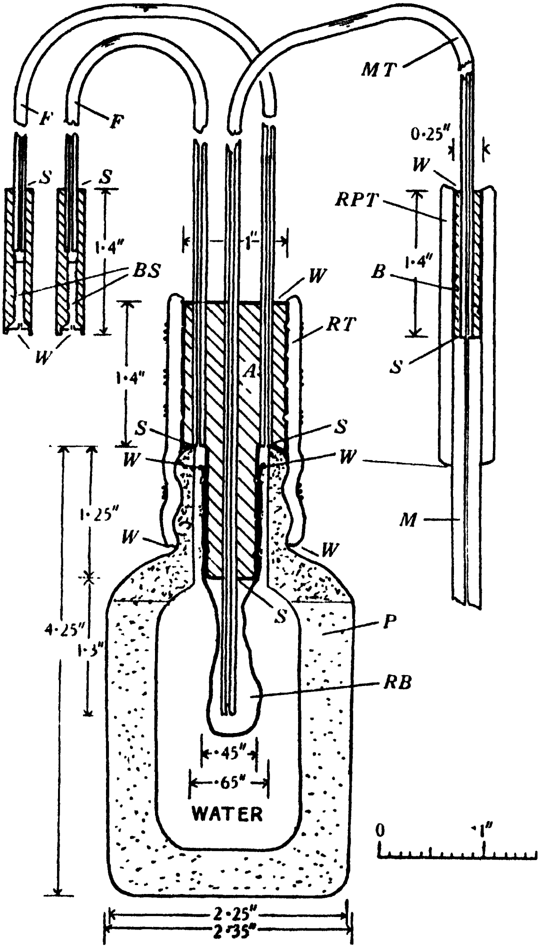


Fig. 7. Details of anti-frost and filling devices of moisture meter.

APPENDIX A.

The design and method of construction of the moisture meter that have been found to give most satisfactory results under field conditions are as follows:

A special thick-walled round porous pot *P* (Text-figs. 1 and 7) is connected by means of a brass adapter *A* to a $\frac{1}{8}$ in. copper tube *MT*. (For a depth of 3 ft., a 10 ft. length of tube is required.) An absolutely airtight connection is essential, and this is secured by making the join with thick rubber tubing *RT*, wired on, and by sealing over the edges with pressure wax (at points marked *W*). The copper tube leads to a mercury manometer *M*, and is connected to it by a brass bush *B* and $\frac{1}{8}$ in. bore rubber pressure tubing *RPT*. The joints are sealed with wax as before. The copper-brass joints are soldered at points marked *S* (Text-fig. 7). The manometer tube dips into a cup of mercury *C*, and is secured to the wooden back (which is recessed to take the rubber join) with a brass clip at the top and two countersunk headed brass screws *CS*, which do not obscure the readings, near the base.

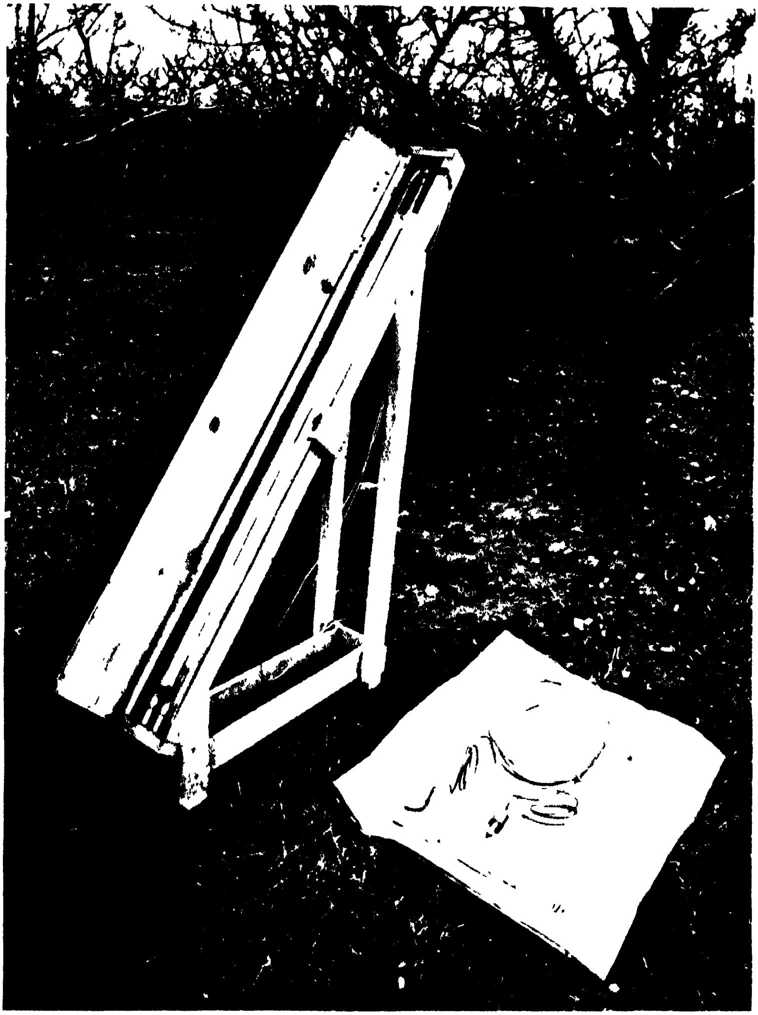
In order to obtain a porous pot of the required thickness and density a special mixture of the material known as "stoneware" is used.

The arrangement of the simple model for laboratory tests (Text-fig. 1) has already been discussed in the text (p. 329).

In the field model a rather more complicated layout is necessary. A sloping wooden stand is used for the manometer, as shown in Pl. VII. This has the double effect of making the manometer easier to read, and magnifying the mercury movements. A hinged lid protects the manometer from the weather.

A special device is used to avoid freezing of the water in the main tube, and also to prevent the air which is inevitably dissolved in the soil water from entering the tube and causing inaccurate readings. This consists of an ordinary toy rubber balloon *RB* (Text-fig. 7) which is placed over the end of the adapter *A* inside the porous pot. The tube and balloon are filled with a 50 per cent. glycerine solution (previously boiled to remove air) which effectively prevents freezing. The balloon is fitted almost collapsed, so that as water is drawn from the porous pot the walls of the balloon are drawn outwards, and the pull is transmitted to the mercury manometer. The porous pot itself must be filled with water, but it is normally safe from freezing on account of its depth in the soil.

Two filling tubes *F* are soldered into the adapter *A*. They are $\frac{1}{8}$ in. diameter and of suitable length to reach the soil surface, with about 2 ft. extra. Screw stoppers *BS* are fitted to the ends, as shown in Text-fig. 7. These filling tubes make the initial filling easier, and also make it possible to remove periodically any air which gets into the porous pot dissolved in the soil water. Also should extreme drought conditions cause the film of water in even the thick porous pots to break, the instrument



Moisture meter installed in the orchard. The two manometers in the casing are connected to pots at different depths. A porous pot and main tube (with filling tubes) are lying on the paper.

can be refilled through these tubes without disturbing the rest of the instrument. When filling, the screw stoppers of both tubes are removed, and water is poured into one tube by means of a rubber tube attached to a funnel. The air escapes from the other tube until the pot and both tubes are filled with water. The screws are then replaced, and their heads dried and waxed over.

The complete instrument is being placed on the market by Messrs A. Gallenkamp and Co. Ltd., Sun Street, London, E.C. 2. A more robust form using a Bourdon tube vacuum gauge in place of the mercury manometer is also being manufactured.

Installing the meter.

The porous pot is buried in the soil at the required depth. The manometer is placed 2 or 3 ft. away, and in such a direction that it does not keep rain off the ground round the pot more than is inevitable. As small a hole as possible is made for the pot, and each layer of soil is kept separate and replaced in its correct position after the pot has been placed in position. The soil is compressed to occupy the same volume as before. A time when the soil is not too wet should be chosen for installing the meter.

The projecting ends of the filling tubes are carried along a few inches below the soil surface to a marked spot about 2 ft. from the pot, and are normally left buried there so that they are protected from heat and frost.

The danger of drainage channels developing round the tubes of the meter and at the sides of the hole is avoided as far as possible by careful ramming. If desired, flanges can be soldered to the tubes just below the soil surface. It is an advantage to set the porous pot in a cavity scooped in the side of the hole dug for its reception, so that it is mainly surrounded by normal soil. Alternatively, an auger of exactly the right diameter can be used for setting the pot.

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(*Received March* 11*th*, 1935.)

THE PRE-NATAL DEVELOPMENT OF THE COAT OF THE NEW ZEALAND ROMNEY LAMB.

BY NANCY GALPIN, M.Sc.

(Massey Agricultural College, Palmerston North, New Zealand.)

(With Ten Text-figures.)

CONTENTS.

		PAGE
I.	Introduction	344
II.	Material and methods	344
III.	Development of the coat	347
IV.	Discussion	356
V.	Summary	358
	References	360

I. INTRODUCTION.

THE classification of fibre type arrays (Dry (2)) is based on postulated pre-natal events. Very little is known about the pre-natal development of the coat of the sheep, and the work that has been done has been of histological (3, 6) rather than of morphological importance. It was therefore desirable to study the development of the coat of the sheep with a view to interpreting post-natal morphological expressions from pre-natal events.

II. MATERIAL AND METHODS.

The material consisted of a series of dated New Zealand Romney foetuses from the Massey Agricultural College flock and a number of undated ones from various sources.

Macroscopic examination was carried out with the aid of a hand lens, and more detailed knowledge was gained by taking snippets of skin from definite areas and mounting these for microscopic examination.

The earlier foetuses were fixed immediately they were taken from the uterus by immersion in a formol-alcohol bath—5 per cent. formalin, 30 per cent. alcohol; this bath was changed after 24 hours and the foetuses were stored in a second similar bath. This was found to be a satisfactory method of preserving the earlier foetuses—the tissues remaining in a suitably stretched condition. The later foetuses—from 112 days—were immersed in a 5 per cent. formalin bath instead of the formol-alcohol one. They were changed to a similar solution in which they were stored in the same way as for the earlier ones.

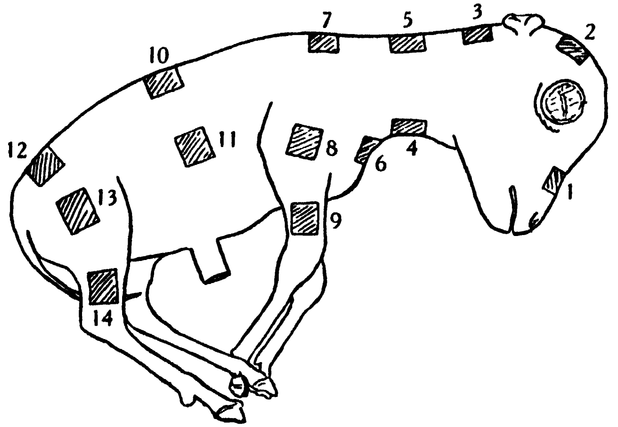

Fig. 1. Diagram of foetus to indicate positions from which skin snippets were taken. 1, nose; 2, poll; 3, neck; 4, ventral neck; 5, anterior withers; 6, shoulder point; 7, withers; 8, h.-r.j.; 9, forearm; 10, back; 11, side; 12, superior ileum; 13, hip joint; 14, britch.

Skin samples were regularly taken from the following positions:

(1) *Nose.* Across the bridge of the nose just anterior to the eyes.

(2) *Poll.* On the mid-dorsal line between the ears and the positions of the horns.

(3) *Neck.* On the mid-dorsal line half-way between the ears and the last cervical vertebra.

(4) *Ventral neck.* On the mid-ventral line comparable to the anterior withers position.

(5) *Anterior withers.* On the mid-dorsal line about the level of the sixth cervical vertebra.

(6) *Shoulder point.* On the point of the right shoulder.

(7) *Withers.* On the mid-dorsal line level with the fifth rib.

(8) *Humero-radial joint.* Ventro-lateral to the withers, on the right side, in a line with the lateral aspect of the shoulder point and slightly anterior to the elbow.

(9) *Forearm.* Half-way down the right forearm.

(10) *Back.* On the mid-dorsal line level with the last rib.

(11) *Side.* On the right side at the distal end of the last rib.

(12) *Superior ileum.* On the mid-dorsal line on the medial angles of the ilea.

(13) *Hip joint.* Ventro-lateral to the superior ileum position at the junction of the femur and pelvic bones (acetabulum).

(14) *Britch.* Immediately posterior to the stifle joint and midway across the thigh.

In addition skin snippets were taken from many other positions, but not from every foetus. The skin snippets were stained with borax carmine, cleared and mounted in xylol balsam. This staining proved most effective, the follicles staining a deeper red than the skin generally.

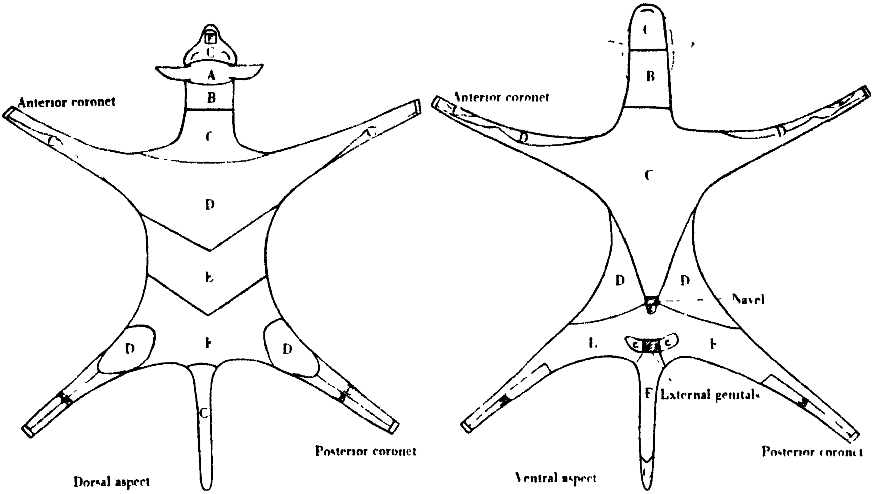

Fig. 2. Dorsal and ventral aspects of the skin, laid flat, to show the positions of the areas referred to in the text. The sensory follicles around eyes, mouth, ears and nostrils and those of the anterior and posterior coronary regions do not belong to any area.

Examination of skin snippets revealed the fact that the body naturally divided itself into regions that developed at different times. These regions were not all contiguous, and for the purposes of description all regions that develop at the same time have been grouped together and termed an area. There are six of these areas and they have been described as areas A, B, C, D, E and F, respectively—area A including the regions that develop first, area F those that develop last. These areas do not include the sensory follicles around the eyes, mouth, ears and nostrils, nor those on the anterior or posterior coronary regions; these are referred to by name when mentioned in the text.

The areas include the following regions (see Fig. 2):

Area A. The poll region.

Area B. The anterior neck region—dorsal and ventral. The posterior aspect of the hind-limb inferior to the hock joint.

Area C. The dorsal and ventral portions of the posterior neck region. The carpal areas and the posterior aspect of the fore-limb. The large ventral region between the fore-limb extending laterally to the anterior coronary region and mid-ventrally a short distance behind the navel. The small regions on either side of the external genital organs. The dorsal surface of the tail and the posterior third of the ventral surface. The face region. The ventral aspect of the lower jaw.

Area D. The withers region extending laterally to the anterior coronary region and mid-dorsally to a short distance anterior to the last rib. The cranial face of the fore-limb inferior to the carpal region. The lateral regions of the ventral surface as far posterior as the navel. The britch region.

Area E. The dorsal and lateral regions of the back. The lateral limb regions inferior to the britch. The ventral surface posterior to the navel and extending down the limbs.

Area F. The ventral anterior two-thirds of the tail. The dorsal surface of the hind-quarters posterior to E area. The bridge of the nose.

III. DEVELOPMENT OF THE COAT.

The development of the coat of the New Zealand Romney lamb was found to correspond in the main with that described by Wildman (6) for certain British breeds. The principle of crown-rump measurement as normally employed has been used in this paper and has been given for each foetus along with its age.

It will be noticed, however, that considerable importance was attached to the stage of development termed "trio" (4, 5), which Wildman (6) made reference to but did not stress. Further, reference has been made to a "nine" stage—a derivative of the trio stage—which has been considered of equal importance to the trio stage; this stage when completed might well be mistaken for a complete trio stage—description appears later in the text (see Figs. 6 and 10). This nine stage was not mentioned by Wildman (6) in his description of British breeds, but along with the trio stage it has been found a very satisfactory stage by which to date foetuses approximately. A discussion as to its importance will be given later.

At 42 *days*. The earliest dated foetus taken was a 42-day one (6 cm. crown-rump measurement). Follicles were present around the eyes, nostrils and mouth, both upper and lower lips; these were the follicles of tactile fibres. The follicles around the eyes were very prominent, being evident as large white rounded spots. No follicles were to be seen elsewhere on the body.

At 49 *days* (Fig. 3). On the 49-day foetus (7½–8 cm. crown-rump measurement) a few scattered single follicles had appeared on the poll (A area), and isolated ones on the anterior portions of the neck (B area). Posterior and anterior coronary regions both had follicles. With the exception of sensory follicles mentioned as occurring in the 42-day foetus on no other regions of the body could follicles be found.

At 56 *days* (Figs. 3 and 4). The 56-day foetus (11 cm. crown-rump measurement) had isolated follicles over most of the body, but they were more difficult to find on the E and F areas than elsewhere. On the A areas it was possible to distinguish two types of first follicles, *X* being the term applied to the larger and older, and *Y* to the later and smaller of these (see Figs. 3 and 4). The follicles were numerous on the A area. The B area had a number of follicles but it was not possible to distinguish *X* and *Y* follicles. The B area was, however, at a more advanced stage than the other areas, the most noticeable differences being the greater size of the follicles and the greater numbers.

At 64 *days* (Figs. 3, 4 and 5). At 64 days the foetus was 14 cm. in crown-rump measurement, and had follicles all over the body. Variation in density was quite marked, density here signifying the number of follicles per unit area, that is, population of fibres per unit area of skin surface (6). On the A area, the *X* and *Y* follicles were numerous, and the larger *X* follicles had now two small follicles, one on either side, giving the trio arrangement mentioned previously (Fig. 5). These small follicles have for convenience been termed *x* and *y*, so that the trios may be written *xXx* and *yYy*. The *X* and *Y* follicles of the A area had arranged themselves in lines. The trio grouping did not disturb this arrangement (see Fig. 5). On the B area both *X* and *Y* follicles were apparent but no trio grouping had appeared as yet. On the C area large *X* follicles and small *Y* ones were found; D area had *X* follicles with *Y* ones just appearing; E and F areas had isolated follicles scattered over the field. The follicles on the E area, however, were slightly more pronounced than those on the F area. Linear arrangement of follicles was to be seen on the A and B areas, but nowhere else on the body.

At 68 *days* (Figs. 3, 4, 5 and 6). The 68-day foetus (15 cm. crown-rump measurement) had all the follicles of the A area in trio groups *xXx* and *yYy*. The B area had *X* and *Y* follicles which were assuming a linear distribution—appearing much the same as the poll did at 56 days. The C area was similar, but the follicles were smaller, especially, the *Y* ones.

The D area had the same follicles present and the same follicular arrangement as the D and C areas, but the follicles were noticeably smaller. Only

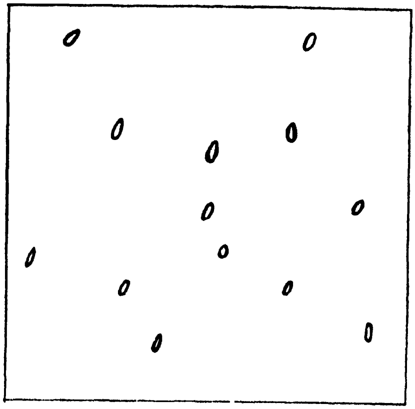

Fig. 3.

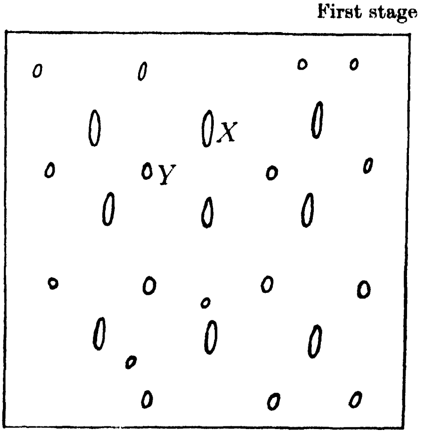

Fig. 4.

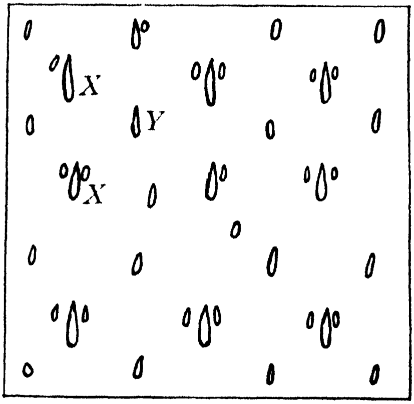

Fig. 5.

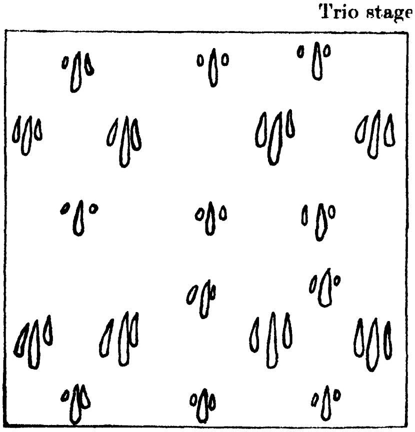

Fig. 6.

Figs. 3–6. Diagrams to illustrate earlier stages of follicle distribution in the pre-natal skin.
Fig. 3. First follicles just appearing.
Fig. 4. Two types of follicles; the larger ones are *X* follicles, the smaller ones *Y* follicles.
Fig. 5. Shows commencement of trio stage. The *X* follicles show trio arrangement, but the *Y* follicles have not yet reached this stage. Note the linear arrangement.
Fig. 6. Complete trio stage.

X follicles were found on the F area, while *X* and suggestions of *Y* follicles were to be seen on the E area.

At 72 *days* (Figs. 4, 5, 6 and 7). The 72-day foetus (19–20 cm. crown-rump measurement) showed the trio arrangement disappearing on the A area. This was apparently due to the fact that at or about the 68-day stage X and Y follicles on this area had completed their earliest development and were about to commence fibre growth. When the 72-day stage is reached the x and y follicles of the trio have also nearly completed development and are about to commence fibre growth, so that at 72 days all follicles of the trio—xXx and yYy—look much the same. At this stage it was impossible to differentiate between trio groups of the xXx and yYy types. Grouping in threes was, however, still detectable (Fig. 7). Among the follicles of the trio grouping a number of new small follicles were found. They did not necessarily appear close to the middle or oldest follicles of the trio group but were situated indiscriminately beside either X or x, or Y or y. In the B area all the follicles were at the trio stage of development; on the C area the X and most of the Y follicles had reached the trio stage of development, while on the D area only the X follicles had reached this stage. The number of follicles on the E and F areas had increased; the E area showed a linear follicular arrangement, while on the F area there were still a number of Y follicles just appearing.

At this stage the follicles around the eyes and nostrils and on the upper and lower lips were very prominent, and tactile fibres were nearly ready to burst through the skin.

At 76 *days* (Figs. 4, 5, 6, 7 and 8). The next foetus was 76 days old (20 cm. crown-rump measurement). The A area now retained only a suggestion of the trio arrangement, it being impossible to distinguish between any of the xXx or yYy follicles of the trio except by their position. Additional new follicles; $x'y'$, were developing (Fig. 8) rapidly beside the various members of the trio. The B and C areas had completed their trio stage of development (Figs. 6 and 7). The B area had the trio stage disappearing, and odd new x' and y' follicles were appearing as on the poll at 72 days (Fig. 7). The C area, though not so advanced as that of B, also had suggestions of the new x' and y' follicles. The D area had a few yYy trios to be completed (Fig. 5); the F area follicles were linearly arranged (Fig. 4).

At 83 *days* (Figs. 6, 7, 8 and 9). At 83 days the foetus (22–23 cm. crown-rump measurement) had the tactile eyelash fibres through the skin. The A area on casual inspection were much the same as at 76 days, but closer examination revealed further development of x' and y' follicles about the old xXx and yYy trio groups. These new follicles were becoming arranged in groups about either the X or x, the Y or y follicles of the original trio group in a manner suggestive of the original trio grouping which had now completely disappeared. This was the foreshadowing of the nine grouping—which was due to each of the follicles of the trio group becoming the central follicle of a group of three follicles—making each trio the forerunner of a nine group. At this stage groups of seven and eight could be found. The x' follicles were still small (see Fig. 9). On the B, C and D areas the trio arrangement of follicles was no longer obvious, but close inspection revealed the remains of such grouping. New x' and y' follicles were appearing, B and C areas showing groups of five and six follicles. An odd group of seven might be detected on the B area. On the E area the suggestion of a trio arrangement still persisted, but now x' and y' follicles were appearing. The F area were in the late stage of trio development, the follicles of x and y type being of the same size as the X and Y.

At 90 *days* (Figs. 7, 8, 9 and 10). On the 90-day foetus (29–30 cm. crown-rump measurement) the fibres round the nostrils, and on upper and lower lips, the horn and posterior coronet regions, had all burst through the skin, while the hairs around the eyes were quite long.

The follicles of the A area were now grouped in nines, each nine originating from an original trio group. There were thus three groups of three, one about the X follicle of the trio group and one about each x follicle. This grouping $x'xx'$, $x'Xx'$, $x'xx'$ (which may very easily be mistaken for an advanced trio grouping) showed on closer inspection the nine grouping and the origin of the group (Fig. 10). In addition new follicles were beginning to appear beside the nine-stage follicles. Some of the follicles on this area were ready to burst through the skin. B, C and D areas showed a further increase in the number of x' and y' follicles, the B area having very nearly reached the complete nine-grouping stage. On the E area remains of the trio arrangement could still be detected, but the number of x' and y' follicles was increasing. On the F area, the suggestion of the trio arrangement still persisted though it was rapidly disappearing.

At 97 *days* (Figs. 8, 9 and 10). At 97 days (30 cm. crown-rump measurement) the foetus had quite a number of regions on which the fibres had emerged through the skin. The hairs were quite long on those regions where fibres had been found through the skin at the 90-day stage. The fibres on the anterior coronet region were through.

The fibres had emerged through the skin on the A area and were just piercing it on the B area. The C area showed the completed nine groups, though there were still a few groups of seven and eight to be completed. No complete nine groups occurred on the D area, but there was a number of groups of eight; on the F area the remains of the trio stage could with difficulty still be detected. The E area had a number of groups of five, six and seven follicles, while the F area had the x' follicles appearing, groups of four and five being quite common.

Trio stage

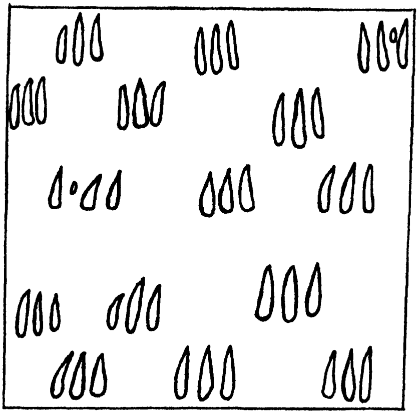

Fig. 7.

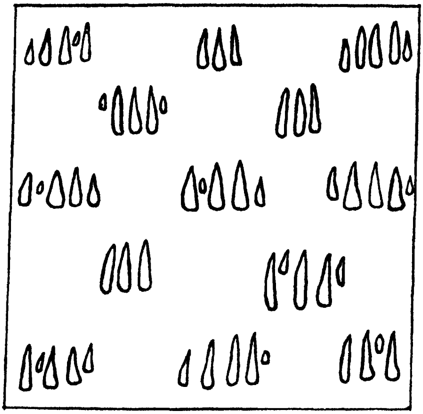

Fig. 8.

Nine stage

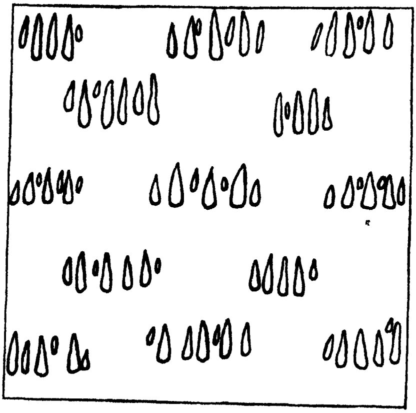

Fig. 9.

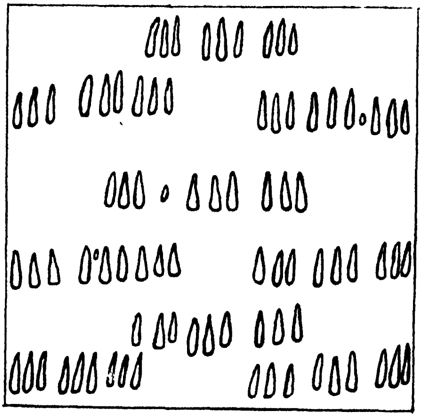

Fig. 10.

Figs. 7–10. To illustrate the later stages of follicle distribution.
Fig. 7. Complete trio stage. Follicles all approximating each other in size.
Figs. 8 and 9. Stages intermediate between the trio and nine stages.
Fig. 10. Complete nine stage. Linear arrangement is still perceptible.

Owing to the sterility of one ewe no foetus intermediate between 97 and 111 days was secured.

At 111 *days.* The next foetus was aged 111 days (32–33 cm. crown-rump measurement). This had fibres through on all areas except E and F.

The fibres of the A and B areas were quite long and had commenced to curl, while on the C area the fibres were through the skin. The D area had the fibres just piercing the skin. On the E area fibres were not through. The nine grouping was complete and some of the follicles were just ready to pierce the skin. On the F area the nine grouping was very nearly complete.

At 122 *days.* The 122-day foetus (40–43 cm. crown-rump measurement) was completely covered with fibres. On the early regions—the A and B area—it was possible to distinguish the halo-hairs and sickle fibres. On the C and D areas suggestions of sickle fibres could be detected, while on the E and F areas the fibres were short and straight. This ewe had triplets, and the wool characters of the lambs were somewhat different, two having a large number of halo-hairs while the third had very few.

At 127 *days.* The 127-day foetus (47–50 cm. crown-rump measurement) had relatively long fibres present. On the earlier areas—A, B and C—there was quite a marked curl effect, the region about the shoulder point being the most tightly curled. Pre-curly-tip fibres were easily recognisable from A, B and C areas, and also some curly-tip ones were distinguishable. As well as these there were some fine, straight, short fibres not able to be distinguished as yet as belonging to any definite fibre type. On the E and F areas, all the fibres were uncurled and were not able to be sorted into definite fibre types. On the earlier regions it was possible at this stage to find representations of all fibre types, except histerotrichs.

At full term (146 days average). No dated foetuses were secured older than 127 days, but some lambs that died at birth were secured. These were from 50 to 54 cm. crown-rump measurement. The wool was found to be curled on all regions, the halo-hairs standing up rather stiffly. The fibres had grown considerably longer in the time intervening between 127 days and birth. Samples taken from various regions on these when sorted gave halo-hairs, super-sickle, sickle and curly-tip fibres and a few short fibres which might have been histerotrichs but in all probability were late curly-tip ones.

Undated foetuses. From the dated material in my possession no definite information could be gathered as to whether the fibres on the E or F areas pierced the skin first. From the relative times at which fibres in the two areas began to grow it was surmised that in E area they pierced slightly in advance of F. Undated material confirmed this. An undated foetus (33 cm. crown-rump measurement) had fibres in the E areas just piercing the skin, while those in F area had not emerged. Two other foetuses (33–36 cm. crown-rump measurement) had their E areas with fibres well through and their F areas with just a few scattered fibres piercing the skin. In addition there were a number of white papilla-like spots which were follicles with the fibres just ready to pierce the skin.

Another foetus of 32 cm. crown-rump measurement showed the mid-dorsal line of the E area in advance of the side regions of the same area. This was also found on the 33–36 cm. long foetuses which had only a few fibres piercing the skin on the F area, the number of fibres through on the mid-dorsal line being greater than elsewhere.

One whole skin of an undated foetus (21 cm. crown-rump measurement) was stained with borax carmine and cleared in cedar-wood oil. On this foetus the trio arrangement could still be detected on the D area, while on the B and C areas the new follicles coming in were beginning to mask the arrangement. On E area the trio grouping was obvious, but new x' and y' follicles were appearing. The F area was still at the trio stage.

Study of the skins of the dated series suggests that the approximate age of this foetus was 83 days. Study of the arrangement of the follicles revealed a linear arrangement similar to that figured for the Masham foetus in Wildman's paper ((6), p. 267).

Examination of the undated foetus illustrates the fact that fibres burst through the skin in the order that they were laid down—that is, in accordance with the directional currents—halo-hairs appearing first, followed by sickle fibres, curly-tips, and last of all histerotrichs.

In the account of the development of the coat it will be noted that after the nine stage there was no further mention of development in threes. Odd follicles were mentioned as appearing alongside members of the nine stage which may suggest that a further stage—a twenty-seven stage—was possible. From post-natal observations, however, this was unlikely. In a paper now being prepared for publication will be found an account of the distribution in the coat of the Romney lamb of the pre-curly-tips, that is, the halo-sickle group of fibres. It was found that the earlier an area commenced follicle development, the more numerous were the pre-curly-tip fibres. The proportion of pre-curly-tip fibres was never more than 33 per cent. of all fibres; on the poll region they usually approximated to this number. We thus find that pre-curly-tips constituted about a third of the coat. The remaining two-thirds were curly-tips and a few histerotrichs. From this we might assume that the pre-curly-tips were from the trio stage follicles and the curly-tips from the additional follicles constituting the nine stage. The histerotrichs might be from

Table I. *Showing the stage of development of different areas at different ages.*

Age of foetus (days)	Area A	Area B	Area C	Area D	Area E	Area F
42	—	—	—	—	—	—
49	X	—	—	—	—	—
56	X and Y	X	X	X	X	—
64	xXx and Y	X and Y	X and Y	X and Y	X	X
68	xXx and yYy (trio stage)	X and Y	X and Y	X and Y	X and Y	X
72	xXx and yYy (late trio stage)	xXx and yYy (trio stage)	xXx and Y and yYy	xXx and Y	X and Y	X and Y
76	$xXx'x$ and $y'yYy'y$	xXx and yYy (late trio stage)	xXx and yYy (trio stage)	xXx and yYy and odd Y (trio stage)	xXx and Y	X and Y
83	$x'xx'Xx'x'xx'$ and $y'yy'Yy'yy'$ (trio gone)	$x'xXx'xx'$ and $yy'Yy'yy'$ (trio gone)	$xx'Xx'x$ and $y'yYy'yy'$ (trio gone)	$xXx'x$ and $yy'Yyy'$ (trio disappearing)	$xXx'x$ and yYy (late trio stage)	xXx and yYy (late trio stage)
90	$x'xx'$, $x'Xx'$, $x'xx'$ and $y'yy'$, $y'Yy'$, $y'yy'$ (nine stage)	$x'xx'$, $x'Xx'$, $x'xx'$ and $y'yy'$, $y'Yy'$, $y'yy'$	$x'xx'Xx'x'x$ and $y'yy'Yy'y'yy'$	$x'xx'Xx'x$ and $y'yy'Yy'yy'$ (trio gone)	$xx'Xx'x$ and $yYy'y$ (trio still detectable)	$xXx'x$ and $yYy'y$ (trio disappearing)
97	Fibres through the skin	Fibres piercing the skin	$x'xx'$, $x'Xx'$, $x'xx'$ and $y'yy'$. $y'Yy'$. $y'yy'$ (nine stage)	$xx'Xx'x'xx'$ and $yy'y'Yy'y'yy'$	$xx'Xx'xx'$ and $yy'Yy'y'yy'$ (trio gone)	$xXx'x$ and $yYy'y'y$ (trio gone)
111	Fibres begun to curl	Fibres begun to curl	Fibres through the skin	Fibres piercing the skin	$x'xx'$, $x'Xx'$, $x'xx'$ and $y'yy'$, $y'Yy'$, $y'yy'$ (nine stage)	$x'xx'x'Xx'xx'$ and $y'yy'$, $y'Yy'$, $y'yy'$
122	Halo-hairs and sickle fibres recognisable		Fibres begun to curl, sickles present	Fibres begun to curl, sickles present	Straight fibres	Straight fibres
127	All pre-curly-tip and some curly-tip fibres recognisable		Pre-curly-tip and a few curly-tip fibres recognisable		—	Straight fibres

the few extra follicles appearing after the nine stage. If a twenty-seven stage appeared one would expect that the histerotrichs would be twice as numerous as the pre-curly-tips and curly-tips together.

Further, if any stage later than a nine stage occurred, the post-natal density of the Romney—not a very densely-woolled sheep—would necessarily be very high, or the sheep very much larger than it is. It is conceivable, however, that on a dense-woolled sheep such as the Merino there might be a complete twenty-seven stage of development.

A point of considerable interest was the comparatively long time taken between the laying down of the follicle and the piercing of the skin by the fibre. On the poll region, the first follicles are laid down at about 7 weeks, while the first fibres do not pierce the skin surface till after 13 weeks. The time from the initiation of the follicle till it had attained its full length was between 2½ and 3 weeks. The remaining period—2½–3 weeks—was taken up with the formation of the fibre.

Rudall (in a personal communication) mentioned that it took over 2 weeks for the regeneration of a fibre.

When the lapse of time between follicle initiation and the fibre piercing the skin was considered, it was not inconceivable that there was a delay between the causal event determining the pre-natal check and the resultant expression.

Dry (1) in the mouse found that the length of a zigzag fibre could be prophesied within narrow limits by examining the length of the distal segments of some fibres. This indicated that total length was determined at an early stage of development. In the sheep he found expressions that suggested that they were the delayed result of a causal event. Such expressions were the percentage of chalky sickles shed and the nature of the halo-hair successors (Dry, unpublished paper).

The nature of the succession of the first lot of secondary kemp was evidence of a very much delayed result of a past causal event (Dry, unpublished paper).

IV. DISCUSSION.

Before summarising the account of the follicular development, it will be necessary to discuss the trio and nine groupings which have figured somewhat prominently in the above account. The present work emphasises the fundamental importance of the number three, the importance of which in hair arrangement was first recognised by de Meijere (5).

The trio stage of development was referred to by Wildman ((6), p. 272) as occurring on the Down and Scottish Blackface breeds on certain specialised regions. In his account he has not, however, attached the same importance to it as has been done in this account of the New Zealand Romney. On the Romney this trio stage was a definite stage in the pre-natal development of the follicles of the coat. It occurred on all areas, and was a means of determining the stage of development of an area. It was considered that it might be a method of determining the approximate ages of undated foetuses. Examination of undated material, of dated twins, and of two dated foetuses of the same age, suggests that although length and width may vary the stages of development are comparatively constant for the same age. The nine grouping succeeded the trio stage of development, through the development of two follicles, one on either side of each follicle of the trio. This stage might be useful for dating older foetuses.

From this account of the development of trio and nine stages it follows that all the first follicles—the *X* and *Y* follicles—are forerunners of eight additional follicles, giving the following groupings: *xXx* and *yYy* at the trio stage, *x'xx'* and *x'Xx'*, *x'xx'* and *y'yy'*, *y'Yy'*, *y'yy'* at the nine stage.

At the completed nine stage we see additional small follicles appearing which as far as can be ascertained at present do not develop in any regular way, appearing haphazardly beside any of the follicles of the nine group. From both pre- and post-natal observations it was thought that these follicles were not very numerous and never resulted in a twenty-seven stage of development. It was suspected that these follicles were the follicles of the histerotrich fibres. The number of histerotrichs in post-natal material apparently varied considerably, but was not usually high. We found, therefore, that the bulk of the coat was made up of fibres which grow from the follicles of the nine stage.

We had the following pronounced stages in the follicle development:

(1) The foundation, or *X* and *Y* follicle, stage.

(2) The trio stage.

(3) The nine stage.

This arrangement of three successive stages emphasised the fact that the number and distribution of first *X* and *Y* follicles was perhaps the chief factor determining the density of the coat of the adult animal. It was known that at 17 weeks, halo hairs, sickle fibres and curly-tip ones could be seen on the advanced areas. From post-natal work it was known that the proportion of pre-curly-tip fibres per hundred fibres was higher on the advanced than on the later areas (see p. 354). Thirty-three pre-curly-tip fibres per hundred fibres was the highest proportion found.

From this we must assume that in such cases all trio stage follicles produced pre-curly-tip fibres. On the other hand, on some of the later areas we must assume that only the X and Y follicles became pre-curly-tip fibres and not necessarily all of these.

From this we can make the general assumption that there was an original density which varied from position to position.

It has been postulated that the fibre type arrays (2) were due to the action of two variables: (1) the pre-natal check, and (2) the reduction of inherent coarseness. These two variables it was suggested were two expressions of the same kind of balance, the increasing of the varying original (X and Y) densities as opposed to skin expansion at different times, viz. the trio and nine stages. These two variables, however, cannot be separated, but according to the extent of their interaction do we get the varying fibre type arrays. It now seems as if we may regard the pre-natal check as a trio depression[1] and the reduction of inherent coarseness as a nine depression.

The follicles that appear after the nine stage have not been considered as affecting the fundamental fibre type array, being only additional follicles which may or may not comprise all the histerotrich fibres.

It is hoped that future more detailed work on density variation and skin expansion will further test this view.

V. SUMMARY.

For the purpose of describing the development of the Romney lambs' coats it was found convenient to divide the skin into six areas, A, B, C, D, E and F, each being formed by the grouping of all regions that develop at the same time and rate.

These areas are determined by the following methods:

(1) All regions where X and Y follicles begin growth at the same time belong to the same area.

(2) All regions attaining the trio stage of development at the same time belong to the same area.

(3) All regions reaching the nine stage of development at the same time belong to the same area.

(4) All regions on which follicles pierce the skin at the same time belong to the same area.

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[1] The word "depression" is here used in a general way to mean that combination of two or more influences tending to cause the earliest formed follicles to produce coarser fibres than the later formed ones in which, therefore, the potentiality for fibre growth is depressed.

These are the four main stages in the development of the coat and might be termed:

(1) The foundation, or *X* and *Y* follicle, stage.
(2) The trio stage.
(3) The nine stage.
(4) The skin-piercing stage.

These four stages take place on different areas at different times, but never more than three stages could be recognised on a foetus at one time. Further, it was possible to detect marked differences in the extent to which a stage was developed; for example, two areas might both be at the trio stage, but one area might be at an advanced stage and the other at an early stage of trio development. It was a study of these differences in the stages of development of the trio group that was most useful in defining the areas.

From this work we can state: (1) that different areas develop at different times; (2) there is orderly progression in development from A to F areas; (3) there are certain marked stages of development that enable one to judge the approximate age of the foetus by a study of the different stages of follicle development occurring over the foetus at the same time; (4) that each region is a local governor of its own development, this last fact supporting the view that the depression is a local phenomenon.

It has been shown that the first follicles formed were the first from which fibres pierced the skin, and that certain areas were always in advance of other areas in the following order of developmental progress, A, B, C, D, E, then F areas. The time elapsing between the foundation of a follicle and the fibre piercing the skin was between 6 and 7 weeks.

The first fibres (sensory fibres) to come through were on areas carrying sensory hairs. The next regions in which fibres pierce the skin were those around the horns and posterior coronets. The order of development of fibres on areas was found to be the order given above: A, B, C, D, E, and then F.

The particular variety of fibres produced from these first follicles depended upon the pre-natal check (trio depression). In the New Zealand Romney, on all areas except B and C and the withers portions of D they were often halo-hairs; on the finer-woolled breeds, however, they were usually sickle fibres.

The later fibres continue emerging from the skin, and what they become depends upon the time and extent of the reducing of inherent coarseness (nine depression).

In future work it is hoped to correlate these results with post-natal growth. It is proposed to test the theory that the fibre type array is determined by the original density of X and Y and its relationship to skin expansion.

I wish to acknowledge my indebtedness to Dr F. Dry, Prof. W. C. Miller and Mr K. M. Rudall for their helpful criticisms. My thanks are also due to the donors of the Farmers' Union Scholarship for the opportunity to carry out this investigation.

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(*Received February* 15*th*, 1935.)

INVESTIGATIONS IN CROP HUSBANDRY.

II. ON THE AGE OF SEED BEANS.

By F. H. GARNER, M.A. and H. G. SANDERS, M.A., Ph.D.

(School of Agriculture, Cambridge.)

Introduction.

Age of seed has attracted considerable attention from botanists and agriculturalists for many years. Many crops have been studied, and the present knowledge both of the physiological and agricultural aspects of age of seed is fairly extensive. The field bean, however, has been neglected, despite the fact that the age problem is of particular agricultural interest in the case of this crop. The bean crop must be cut whilst the majority of the plant is still in active growth, otherwise the beans will shell from the lower (the larger) pods; in this respect the bean and other leguminous crops are sharply differentiated from cereals. This relative immaturity at the time of cutting has led, in practice, to delay in threshing so that the maturation processes may be completed, for experience has shown that beans are unsafe as a food for stock if used within four or five months of harvesting; this is reflected in the considerable difference in price between old and new beans throughout the autumn. It may be that this price difference is partly responsible for the fact that some farmers thresh out some new beans especially for seed. There is, however, considerable variation in practice among farmers in this connection, for others will only use old beans for seed, and actually defer threshing some of their beans for twelve months in order to have a supply of old beans for sowing. Yet other farmers, uncertain as to the relative merits of new and old seed, make a practice of sowing some of their bean area with the former and some with the latter.

Where so much diversity of practice exists it is desirable that further knowledge should be sought, and hence the writers have carried out field tests during the last three years to compare old and new seed beans; in this respect old must be taken to refer to seed harvested 12–15 months before sowing. In the year 1931–2 an exploratory experiment was conducted to gain experience of handling bean plots, and to ascertain the optimum sampling unit and the best numerical observations to make during development. In the following two years more extensive experiments, with adequate replications, were carried out with new and old beans of two different strains, one being from Northamptonshire and one being a selection grown in Cambridgeshire. This paper is not concerned with the comparison of the strains, but it will be appreciated that their inclusion gave a double comparison of age in each year. In 1933–4 there were in fact four comparisons, because new and old seed of each strain were sown at two different seed rates; on no occasion was there any interaction between seed rate and variety, and consequently the results of the different seed rates are not shown separately.

In the first and third years the experiments were carried out on very heavy gault clay, whilst in the second year the site was on heavy loam. The aim was to treat the plots normally throughout; they were, however, hoed by hand rather than by horse to avoid disturbing pegs.

LABORATORY STUDIES.

There is a very obvious difference between samples of old and new beans of the same strain. Old beans are much smaller, drier, darker in colour and are generally more damaged than new beans.

Table I. *Results from laboratory examination of samples of seed beans.*

		1931–2	1932–3		1933–4	
	Seed	Cambridge	Northants	Cambridge	Northants	Cambridge
Weight of 1000 whole beans in gm.	Old	540·3	476·8	522·7	637·8	598·8
	New	571·5	571·4	637·8	874·1	615·0
Weight of broken beans as a percentage of the sample	Old	4·95	4·76	1·90	7·00	4·13
	New	4·02	1·79	0·84	7·29	2·63
Laboratory germination of unbroken seed:						
Percentage after 7 days	Old	71·5	96·0	78·7	96·0	96·8
	New	78·5	98·0	98·7	80·0	97·6
Percentage after 21 days	Old	93·5	96·0	84·0	97·6	99·2
	New	88·5	98·0	98·7	97·6	98·4

In each year careful samples were drawn from the sacks of seed to be used on the field plots, and the samples were examined in duplicate at the Laboratory; certain of the results obtained are given in Table I. The figures for thousand-bean-weight demonstrate the size difference between old and new beans; this difference was most marked in the case of the Northamptonshire beans for 1933–4 (where it amounted to 37 per cent.). The proportion of broken beans was not high in any case, but with one exception was rather greater in the old than in the new seed. The percentage germination was satisfactory in all cases; there were some slight irregularities, but no constant difference between the two ages of seed. It may be concluded that in all cases the seed was well up to the standard of that used throughout the country.

FIELD STUDIES.

(*a*) *Preliminary year*, 1931–2.

The first year's trial can only be regarded as a rough one, its main object having been to provide experience in handling the crop experimentally. The layout consisted of only four blocks of two plots each, and from each plot thirty-two samples were taken (two at random distances along each of sixteen rows). A sample consisted of a 1 yard length of drill row, but separate figures were obtained for each foot of the yard; it was thus possible to study the distributions given by lengths of 1, 2 and 3 ft. If a unit be judged by the normality of the distribution it gives, then it appears that 3 ft. of drill row is the minimum unit for beans. Such a unit appears to be the least possible because it gives distributions only just normal; in testing the goodness of fit of the normal curve the values of P obtained were 0·085 for new beans and 0·049 for old beans. It so happens that the yard unit gives means not appreciably less than those given by foot units in the case of cereal crops.

The results of this experiment were largely influenced by a difference in seed rate. The same drill setting was used for each type of seed and the weight of old beans sown was 19 per cent. greater (comprising 28 per cent. more beans) than the weight sown of new beans. The effect of this difference in seed rate was visible throughout the experiment. The old seed gave 18 per cent. more plants, 17 per cent. more stems at harvest, 9 per cent. more pods, 11 per cent. more beans and 7 per cent. greater weight of beans than the new seed. From these figures no satisfactory comparison of old and new seed was possible because the inadequate replication in the experiment precluded the use of the covariance method of statistical analysis. The experiment was useful, however, in demonstrating the importance of varying the drill setting so that equal numbers of old and new beans might be sown per acre. In addition useful experience of handling the crop was obtained. Sampling units should consist of at least 1 yard of drill row, and with that length the labour of hand shelling, and of counting numbers of pods and numbers of beans, was not found to be prohibitive.

(*b*) *Experimental years*, 1932–3 *and* 1933–4.

The 1932–3 and 1933–4 experiments provided for generous replication of the four kinds of seed (old and new of Cambridgeshire and of Northamptonshire) beans. In the former year the layout was that of simple randomised blocks; in the latter year there were six randomised blocks for a comparison of seed rates (two and three bushels per acre), each of the half-blocks being divided into four plots over which the kinds of seed were randomised. In both cases the experimental area had carried an oat crop in the previous year: the actual dates of drilling the beans were October 27, 1932 and October 7, 1933. In each year the size of plot was approximately 1/70th acre, consisting of a 16 yard length of eight drill rows; in 1933–4 it was considered expedient to discard one row from each side of each plot.

Table II. *Data from sampling units* 1932–3 *and* 1933–4. *Results for new seed expressed as a percentage of old seed.*

	1932–3			1933–4	
	Northants		Cambs	Northants	Cambs
No. of viable seeds sown per acre	101		95	93	105
No. of plants in January	107	×	109	85†	104
Field germination	*106*		*114*	*96*	*97*
No. of stems in March	126‡		117†	91‡	111‡
No. of stems in March corrected for no. of plants in January	*115*	×	*109*	*109*†	*101*
No. of stems in April	119†		112‡	86	107
No. of stems in April corrected for no. of stems in March	*100*		*106*	*94*†	*97*
No. of stems at harvest	125‡		124‡	85†	109
No. of stems at harvest corrected for no. of stems in April	*112*†		*110*†	*96*	*103*
No. of pods at harvest	117	×	109	81†	106
No. of pods corrected for no. of stems at harvest	*91*		*86*†	*94*	*99*
No. of beans at harvest	111		108	79‡	109
No. of beans corrected for no. of pods at harvest	*95*†		*98*	*97*	*104*
Dry weight of beans at harvest	113		109	99	110
Dry weight of beans corrected for no. of beans at harvest	*101*		*101*	*125*‡	*100*

A × between two columns denotes that the difference when the two strains were taken together was significant at 5 per cent. point.

† Difference between old and new seed significant at 5 per cent. point.

‡ Difference between old and new seed significant at 1 per cent. point.

The results shown in Table II were obtained from sample yard lengths. In 1932–3 the whole plots were harvested with results closely parallel to those given by the sampling yard lengths; whole plots were not harvested, however, in 1933–4, and consequently yard lengths afforded the only data. A total of 8 yard lengths was taken in each year, though the method of randomisation differed. For the first year the method was to take 1 yard length from each of the eight drill rows at a random distance, with the restriction that over the whole plot 1 yard length, and only 1 yard length, could occur in each 2 yard distance down the plot; this arrangement ensured a wide scatter of the units over the plot, but could not provide an estimate of the sampling error. For the second year the method was to divide the plot longitudinally and transversely to form quarters, and to take two random yard lengths out of each quarter. In both years the sample yard lengths were pegged at the first germination count, and subsequent observations were taken on these same units.

In order to keep Table II within reasonable limits all results are expressed in the form of "N/O per cent." Where the figures were derived from actual observations they are shown in plain type, but figures obtained after correction by the method of covariance (except for the field germination) are given in italics. The value of these corrected figures has already been discussed (2); in that discussion the validity of using the Z test was mooted, and subsequent research work has provided a correct method (1) which was used in the reduction of these data.

Great efforts were made to adjust the drill for the various kinds of seed, so that equal numbers of each might be sown per acre, but the table shows that only very limited success was achieved, for differences up to 7 per cent. occurred. It was quite impossible to determine the number of seed sown on each plot. It was only possible to calculate the average number in the case of each kind of seed and, of course, this figure could not be used for correction by the method of covariance; the figures for field germination were obtained, therefore, by straight division of the average number of plants in January by the average number of viable seeds sown.

Germination counts were made in December in both years, and in 1932–3 a large difference between new and old seed was discovered, the new seed producing 30 per cent. more plants in both strains. The old seed sown in that experiment had, of course, been harvested in the summer of 1931, which is well remembered as a very wet year. It will be seen all through the Table that the old seed in this year did relatively worse than in the following year, and it may be that conditions when the seed was harvested are more important than age.

New seed consistently produced more early (March) branches; the difference in its favour was significant in three cases out of four, and in the fourth case, whilst it was highly significant in the opposite direction, the same conclusion was indicated after correction for an abnormally low number of January plants. From March to April the difference between old and new seeds tended to become narrower, and the general indication is that new seed produces more stems than old, and tends to produce them earlier.

At harvest the only stems counted were those bearing pods. No definite statement can be made as to whether the number of stems in March, or in April, provides the better measure of the number of pod-bearing stems at harvest; in 1932–3 the March stem count was more closely parallel to the harvest stem count than was the April count, whilst in 1933–4 the reverse was the case. This slight inconsistency is presumably to be explained by soil and season, but it appears justifiable to conclude that it is only the earlier formed stems which produce pods at harvest. For every 100 harvest stems there were approximately 130 stems in March (about the 10th) and approximately 160 stems in April (about the 10th). The conclusion that it is the earlier produced stems which survive to produce pods at harvest is supported by the corrected figures for harvest stems; it will be observed that the significant differences were in favour of new seed, which, as already pointed out, produced the earlier stems. As with number of stems, so with number of pods, new seed produced more than old in three cases out of four, but the correction of pod number by stem number brought out the fact that the stems from old seed were more heavily podded; this occurred in every case and was significant with Cambridgeshire beans in 1932–3. The position as regards actual number of beans and dry weight of beans was not materially different from that in number of pods. The italicised figures show no very great difference in bean number corrected for pod number, but one outstanding difference in size of bean. In 1933–4 the new beans from Northamptonshire were abnormal. It is shown in Table I that the beans in that sample of seed were abnormally large and from Table II it can be seen that the setting of the drill did not allow adequately for this. Consequently the uncorrected figures for Northamptonshire seed in that year are low throughout the table. Therefore, in this case particular value is attached to the corrected figures, which, with one exception, are more in conformity with the other three columns. The exception is in regard to size of bean, for it is seen that this seed produced beans at harvest which were 25 per cent. larger than those produced by the corresponding old seed; it may be emphasised that this difference in size was not due to fewer plants, the correction by covariance allowing for that. The larger size of the original Northamptonshire new seed may probably be explained by the fact that those beans were threshed out from self-sown plants growing in a crop of wheat; that such beans are not always abnormally large is evidenced by the fact that the new beans used in 1931–2 were grown under similar conditions and were not especially large. The main interest in connection with this lot of beans lies in the fact that the large seed produced correspondingly large beans at the following harvest, which would have led to increased yield had the seed rate been properly adjusted. The plots sown with this seed were outstanding all through the season in vigour of growth. This lends support to the claim often made, that large seed germinates better and produces stronger plants, which often yield more heavily than those from small seed.

In 1932–3, after the yard lengths had been removed at harvest, the remainders of the plots were cut and threshed. The grain yields obtained were of the same relative order, new seed giving a 16 per cent. increase with Cambridgeshire beans and 12 per cent. with Northamptonshire beans; the former was significant with $P < 0{\cdot}01$ whilst the latter only just failed to attain significance at the 5 per cent. point. It is very regrettable that in 1933–4 the corresponding plot yields were not obtained, owing to an unfortunate misunderstanding; had the plot figures been available it is very possible that the difference in the case of Cambridgeshire beans would have been found significant.

It has been said that in 1933–4 the experiment included a comparison of seed rates of two and three bushels per acre. Many farmers are of the opinion that two bushels is an adequate seed rate, but actually sow three bushels because they fear depredations by birds; in this particular experiment a considerable loss occurred over all plots from rooks. The result of the comparison was briefly as follows: The 50 per cent. difference in number of seed sown was approximately maintained in all of the early counts of numbers of plants and numbers of stems. The difference was reduced to 40 per cent. with number of harvest stems, and to 20 per cent. with number of pods, number of beans and with yield. ,Despite the low number of replications these differences were easily significant. Although the rate of multiplication was less at the higher seed rate, the increased return obtained under these conditions, as regards bird damage, would be sufficient to show considerable financial profit.

SUMMARY.

1. New and old seed beans have been compared for three years, in the last two of which precise field experiments have been conducted with two different strains.

2. In general new seed gave higher yields than old seed, but the difference was only of the order of 10 per cent. There was some evidence that conditions when the seed was harvested might be more important than age.

3. New seed produced more branches in early spring, and a greater number of pod-bearing stems at harvest; on the other hand, old seed produced stems that were more thickly podded at harvest, and pods that contained a greater number of beans.

4. In one case abnormally large seed produced very vigorous plants, and very large beans at harvest.

The writers wish to acknowledge gratefully their indebtedness to Mr W. S. Mansfield, the Director of the University Farm, Cambridge, for facilities for carrying out the experiments, and to Messrs J. S. Chivers, W. W. S. Gibbard and the late Mr W. Eaden Lilley for co-operating in providing seed. They are also greatly indebted to the graduate students of the School of Agriculture who assisted in carrying out the experiments.

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(*Received April* 1*st*, 1935.)

THE GROWTH, YIELD AND COMPOSITION OF CERTAIN TROPICAL FODDERS.

By D. D. PATERSON, B.Sc., B.Sc. (Agric.).

(The Imperial College of Tropical Agriculture, Trinidad, British West Indies.)

(With Three Text-figures.)

I. Introduction.

The following paper is essentially a sequel to a previous one (1) describing the results of an experiment designed to test the influence of cutting rotation on the growth of Elephant Grass (*Pennisetum purpureum*). In the introduction to the original article, the importance of improvement among tropical live stock was stressed, and it was pointed out that the first step in this direction was a fuller knowledge of the composition and nutritional values of the available foodstuffs. In the tropics, stock rations are largely composed of roughage in one form or another, as seed crops are required for human consumption, and the purchase of concentrates is often looked on as an expensive and unnecessary luxury. Progress must therefore come first of all from an improvement in the quality of the bulk fodders. The Elephant Grass experiment, although it was a small-scale one, proved conclusively that the length of the cutting rotation influences the growth and composition of this fodder grass in a manner that is strikingly similar to its effects on pasture grasses grown in the temperate zone. It was also shown that there is a definite seasonal trend on the yield and nutritive value of the produce. It was thought that these results were of sufficient interest to merit a second and more comprehensive field trial, including several different varieties of fodder, and having appropriate modifications in the cutting rotations. The second trial has now been running for twelve months, and this paper gives a summary of the accumulated data and resultant conclusions.

II. The experiment.

The four cutting rotations selected were:

Series A. Cut every 45 days or 8 times per year.
Series B. Cut every 90 days or 4 times per year.
Series C. Cut every 120 days or 3 times per year.
Series D. Cut every 180 days or twice per year.

These treatments were arranged in a 4 × 4 Latin square, the plot size being 54 × 63 ft. Each of these main plots was divided into three similar sections to accommodate three varieties of fodder grass, namely:

E. Elephant Grass (*Pennisetum purpureum*).
G. Guatemala Grass (*Tripsacum laxum*).
U. Uba Cane (*Saccharum sinense* var. *Uba*).

A brief account of Elephant Grass has been given in the previous paper (1), and an excellent botanical description will be found in (3). Guatemala Grass (4) is at present not widely known as a fodder crop, and its cultivation is mainly restricted to its original home in Central America and to the adjoining countries. It is a medium-sized perennial grass with broad, dark green, slightly pubescent leaves. The culms are erect and the plants may attain a maximum height of from 6 to 8 ft. and produce a very dense mass of herbage. It is a grass which rarely flowers under Trinidad conditions of climate and soil, and from this aspect is particularly useful as a fodder crop. It has been stated (4) that live stock do not relish Guatemala Grass fodder, but the working oxen on the College Farm eat it readily and the analyses show that it is about average in nutrient value. It is a surface feeder, and the majority of the roots are found in the first 6–9 in. of soil. The lateral root spread is wide, and on a 3 × 1½ ft. spacing there is considerable overlap of the roots from adjoining stools. This shallow root system makes the stools rather prone to collapse in windy weather or to be uprooted by the labourers when they are cutting the thicker culms.

Uba is one of the well-known cane varieties which has been distributed over practically the whole of the tropical world (6). It is a thin cane with long internodes and a heavy coating of wax which gives it a peculiar bluish green colour. It may attain a height growth of 10–12 ft., and in consequence, the mature stools tend to dwarf those of Guatemala Grass of the same age. It is popular both for sugar and fodder production, chiefly on account of its hardiness and high-yielding capabilities. It is grown extensively on sugar estates in Trinidad on the less fertile soil types. The herbage provides a palatable stock fodder at any stage in its development, even the mature canes, if chopped, being relished on account of their sweetness. The root system is less spreading than that of the Guatemala Grass, but penetrates much deeper into the ground.

These grasses were planted on subplot units 54 × 21 ft. in size, in rows 3 ft. apart with 1½ ft. between stools. In order to avoid border effects due to differences in growth habit between varieties or cutting rotations, the outside row of plants all round each subplot was cut and discarded at harvest, leaving an effective subplot unit of 51 × 15 ft., consisting of five rows with thirty-four stools in each. The total area under the experiment was approximately 1·3 acres.

As the arrangement of the varieties within the subplots of any one main plot was randomised, the analysis of variance may be accurately applied to the data in assessing the conclusions.

The experiment was laid out on one of the fields of the College Farm which lies on the dividing line between the sandy schist soils of the Northern Range and the heavier alluvial loams and clays of the Northern Plain. It has been described by Hardy (2) as a sandy or gravelly loam, acidic in character and of medium-low nutrient status. As its inherent fertility is poor and as the farm rotation is one designed to give practically continuous cropping, regular applications of a suitable mixture of fertilisers are essential in order to maintain a moderate level of productivity. This is especially true in the case of perennial fodder crops which may yield up to 20 tons dry matter per acre per annum. Therefore, a basal dressing of 15 tons farmyard manure and 2 cwt. sulphate of ammonia per acre was applied to the experimental area before planting. In addition, blood meal at the rate of 300 lb. per acre was broadcast on all the plots at three-monthly intervals, *i.e.* after each harvest of series B. It was calculated that this periodic light application of an organic fertiliser would be sufficient to ensure comparable data for the successive harvests, and yet would not be so heavy as to mask varietal or rotational differences in yield or composition.

Planting took place from May 15 to 18, 1933. For all three varieties, propagation was obtained by subdivision of old stools, giving sets consisting of a single tiller root section. During the ensuing three months, the blanks were supplied, and two intercultivations with the horse hoe were given to keep down weed growth. A population count taken at the beginning of August showed that the percentage of successes on all plots was over 98 per cent. In consequence, the crop was considered to be sufficiently established, and on August 10 all plots were mown, and the experiment commenced as from this date, and was continued for the succeeding twelve months. In harvesting, the West Indian brushing cutlass was used and the herbage was cut at some 4 in. above ground-level. This is the normal practice with fodder crops grown in Trinidad. Plots were harvested only on dry days after the dew had disappeared, and the green weights were taken in the field.

A summary of these recorded in tons per acre is appended.

	Cutting rotations				
	Series A	Series B	Series C	Series D	*Varietal means*
Elephant Grass	14·7	19·9	16·6	12·4	*15·9*
Guatemala Grass	21·2	27·0	27·2	32·9	*27·6*
Uba Cane	18·0	34·1	34·9	46·4	*33·4*
Rotation means	*18·0*	*27·7*	*26·2*	*30·6*	

III. Comparison of herbage yields.

These figures demonstrate distinct differences between the yields both of the individual cutting rotations and also of the three varieties of grass. Statistics applied to the full data (*vide* Appendix) show that these differences are significant. The comparative results are practically identical with those obtained from consideration of the dry-matter yields, and in consequence, to avoid tedious repetition it is not intended to discuss the above figures at greater length here, but to leave the detailed statistical interpretation to section IV, where the dry-matter yield figures are recorded.

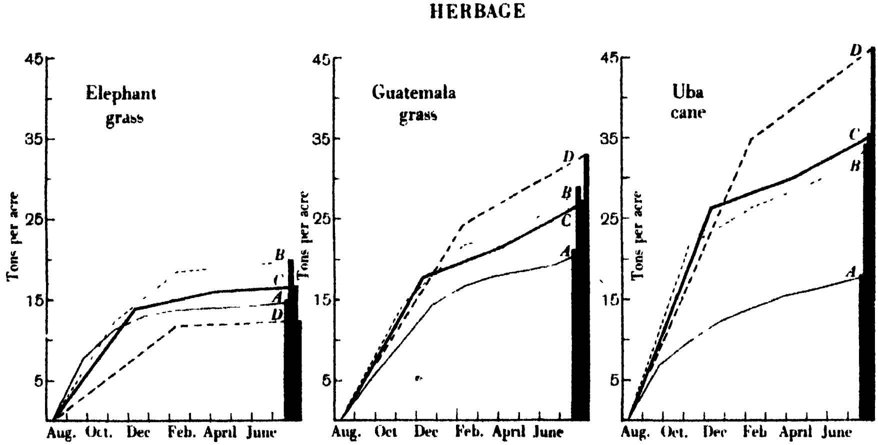

Fig. 1. Showing the relative yields of green herbage from the different fodders and cutting series during the experimental period.

Fig. 1 provides a clear picture of the way in which the final yields were obtained for each variety and cutting rotation. In every case, growth rate has been much greater in the first half of the experimental period. This is explained by the fact that the second half included the dry months, when the lower monthly rainfall causes a much less profuse foliage development in all crops. In this particular year, the dry season extended for a phenomenally long time, and the rains which usually begin in May did not start till July and even then were subnormal. The commencement of the wet season is reflected in the rising trend towards the end of the series A graphs. In most seasons this upgrade would start earlier and be much more pronounced. The prolonged drought is also responsible for making the tonnage per acre per annum in all cases below that which could be expected in a season with a more normal rain distribution.

The relatively poor yields of Elephant Grass are contrary to the findings of Kincaid (4). It is necessary to explain that the plots of this particular fodder were again heavily infected with *Helminthosporium* sp. as first noted in (1). The first visible signs of the fungus appeared on the foliage some 6–8 weeks after planting, and the spread was rapid and progressive, resulting in an intensive parasitism by the end of the experimental period. No reference has been found to show that cultivated crops of Elephant Grass are similarly infected in other countries, and even in Trinidad the attacks tend to be sporadic and occur only in certain seasons. As Elephant Grass is a crop that is normally established in the same field for a number of years, this susceptibility to *Helminthosporium* sp. is sufficient to condemn its use in localities where the fungus is known to be endemic.

IV. SAMPLING AND MOISTURE DETERMINATIONS.

From the nutritional aspect, the yield of dry matter is a much fairer measure of productivity than the weight of green fodder. In order to obtain the necessary data from which to estimate the dry-matter yields, representative samples were taken from each subplot at every harvest to supply material for chemical analysis. These consisted of a minimum of three stools per plot selected at random. The total produce from stools of any one variety and cutting were then bulked and chaffed. The chaffed fodder was next thoroughly mixed and finally two samples of 1 kg. each were selected to provide material for moisture determinations in duplicate. It can be fairly claimed that these samples are truly representative of both the herbage and soil types of which the plots of any particular series are comprised. The mean values for the dry-matter percentages calculated from the duplicate samples are given in Table I.

It is obvious from Table I that two factors are predominant in influencing the dry-matter percentage, namely, the season of the year and the age of the produce. Herbage cut during the period January to

Table I. *Dry-matter percentages.*

		Variety		
Series	Date of harvest	Elephant Grass (E)	Guatemala Grass (G)	Uba Cane (U)
A	Sept. 25	13·38	16·40	14·15
	Nov. 9	12·90	15·75	14·94
	Dec. 25	12·20	14·85	16·95
	Feb. 8	17·12	16·08	16·42
	Mar. 26	23·33	24·54	23·88
	May 10	20·93	24·20	24·22
	June 25	21·90	28·40	27·62
	Aug. 9	17·14	19·67	22·53
Means		*17·36*	*19·99*	*20·21*
B	Nov. 9	21·53	16·45	16·64
	Feb. 8	20·45	20·40	16·80
	May 10	27·52	30·42	26·45
	Aug. 9	23·52	23·22	25·32
Means		*23·26*	*22·62*	*21·30*
C	Dec. 9	24·35	16·95	18·46
	Apr. 9	31·20	35·38	28·95
	Aug. 9	23·28	23·51	23·66
Means		*26·28*	*25·28*	*23·69*
D	Feb. 8	23·25	22·82	24·75
	Aug. 9	21·23	20·89	21·18
Means		*22·29*	*21·86*	*23·47*

July when the rainfall was comparatively light had a much lower moisture percentage than that obtained in the wet season. Similarly, produce from the longer cutting rotations tends to have a higher dry-matter content than that from plots harvested more frequently. The relative significance of the corresponding values for the three grasses is not so obvious. In order to provide an effective species comparison, the differences between corresponding readings for the three fodders were tabulated, and an analysis of variance carried out on these difference data. This was effective in eliminating seasonal variation from the statistics, and showed that, on a probability of 0·01, the young herbage of Elephant Grass has a moisture percentage some 2–3 per cent. higher than either Guatemala Grass or Uba Cane. These last two are about equal in their dry-matter contents with an average value ranging from 20–25 per cent., depending on the maturity of the herbage when cut.

V. YIELD OF DRY MATTER.

The data in Table I have been used to calculate the dry-matter yields of each plot for each harvest. Table II shows the total yield per plot during the experimental period.

Table II. *Yields of dry matter in lb. per subplot per annum.*

Main plot No.	Series A Variety			*Main plot totals*	Series B Variety			*Main plot totals*	Series C Variety			*Main plot totals*	Series D Variety			*Main plot totals*
	E	G	U		E	G	U		E	G	U		E	G	U	
1	—	—	—	—	187	252	298	*737*	—	—	—	—	—	—	—	—
2	70	133	143	*346*	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	134	194	258	*586*	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	113	325	460	*898*
5	—	—	—	—	—	—	—	—	—	—	—	—	109	277	430	*816*
6	—	—	—	—	—	—	—	—	125	263	341	*729*	—	—	—	—
7	—	—	—	—	143	224	234	*601*	—	—	—	—	—	—	—	—
8	80	146	120	*346*	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	222	246	220	*688*	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	97	293	371	*761*
11	77	154	117	*348*	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	179	248	253	*680*	—	—	—	—	—	—	—	—
13	96	146	115	*357*	—	—	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	163	181	220	*564*	—	—	—	—	—		—	—
15	—	—	—	—	—	—	—		—	—	—	—	133	260	481	*877*
16	—	—	—	—	—	—	—	·	173	190	297	*660*	—	—	—	—
Treatment totals	323	579	495	*1397*	672	905	1005	*2582*	654	893	1116	*2663*	452	1155	1745	*3352*

Variety totals:		
	Elephant Grass	2101 lb.
	Guatemala Grass	3532 ,,
	Uba Cane	4361 ,,
	Grand total	9994 ,,

Analysis of variance of dry-matter yields in lb. per plot.

Factor	Sum of squares	Degrees of freedom	Variance	*Z* by calculation
Total for main plot units	178,213	15	—	—
Rows	570	3	190	—
Columns	2,866	3	955	—
Cutting rotation	164,650	3	54,883	1·7408
Error (*a*)	10,127	6	1,688	—
Total for subplot units	460,970	47	—	—
Fodder Grass	163,388	2	81,694	2·2140
Interaction: cutting rotation × fodder grass	95,929	6	15,988	1·3986
Error (*b*)	23,440	24	976	

The mean squares for treatments are all significant as tested by the 1 per cent. value of *Z*. The proportional error variances may be validly used to compare the yields of dry matter as recorded below in tons per acre. In assessing results a value of *t* for $P = 0{\cdot}05$ has been used throughout.

	Cutting rotations				Varietal means	
	Series A	Series B	Series C	Series D	Tons per acre	Percentage of Uba
Elephant Grass	2·05	4·27	4·17	2·88	3·34	48·3
Guatemala Grass	3·69	5·75	5·68	7·34	5·62	81·2
Uba Cane	3·15	6·39	7·10	11·10	6 93	100·0
Rotation, tons per acre	2·96	5·47	5·65	7·11	—	—
Means, percentage of series D	41·7	77·0	79·5	100·0	—	—

For each of the grasses separately, cutting every 45 days (series A) results in a significant decrease in yield. There is no yield difference between the series B and C plots of any one variety. When these series are compared with series D—the six-month rotation—the Elephant Grass shows a greater tonnage, but the Guatemala Grass and Uba Cane a smaller tonnage of dry matter.

As regards varietal comparisons, it can be definitely stated that for any cutting rotation the Elephant Grass has yielded less than either of the other varieties. As already explained, this is attributed to the heavy *Helminthosporium* infestation on the foliage of this species. The Guatemala Grass and Uba Cane gave approximately the same yields of young herbage. When the cutting rotation is extended beyond the three-month period the Uba Cane tends to outclass the Guatemala Grass. The difference is particularly marked in series D. The superiority of Uba is therefore a direct result of its ability to form canes which weigh heavily but are relatively fibrous and non-nutritious. This aspect is discussed more fully in section VII, where the protein yields are recorded. Fig. 2 effectively illustrates these facts

VI. HEIGHT MEASUREMENTS.

Before every harvest of each subplot, five stools were selected at random and measurements taken to provide an estimate of the height of the produce at any particular season of the year for each variety and each cutting rotation. These measurements were recorded to the nearest 6 in. and represent the height to which the three longest tillers in any selected stool attained at harvest. The mean values over the year for the various treatments were:

Cutting rotations		Fodder Grass	
Series A ...	2·96 ft.	Elephant Grass ...	4·90 ft.
„ B ...	4·80 „	Guatemala Grass ...	4·87 „
„ C ...	5·40 „	Uba Cane	5·38 „
„ D ...	7·05 „		

Statistics applied to the full data prove a progressive increase in height for all the grasses from series A to D. This increase is positively correlated with the yield data, so that the taller the crop at harvest the greater will be the gross yield per annum. If the average figures for all cutting rotations are considered, Uba Cane gives a significantly greater height growth than either Elephant or Guatemala Grass. This is due entirely to its taller habit of growth in series C and D, in which the stools of Uba have begun to cane. In the more frequent cutting rotations where the herbage is less mature at harvest, it has no height advantage over the other grasses.

Just as with the yield data, there is a distinct seasonal trend, the produce in all varieties being much shorter in the dry months than in the wet. The mean values recorded above for each grass and each cutting rotation will vary over a range of approximately ± 30 per cent., depending on the rainfall incidence during the period previous to harvest.

VII. YIELD OF PROTEIN.

The data, as tabulated and discussed in the preceding sections, prove conclusively that the greatest yield of green fodder and dry matter is obtained from the long cutting rotations, with four to six months between successive harvests. From the nutritional aspect the composition of the produce is just as important as the total yield per acre, and, for the feeding of live stock, the most valuable and also the most variable constituent in the produce is protein. The earlier experiment has shown that, the older the herbage, the lower the protein, and the higher the crude fibre percentage, and it is therefore questionable whether the heavy yields from series D in the present experiment will compensate for the loss in nutritive value. The samples from which the dry-matter percentages were estimated were therefore used to provide material for nitrogen determinations. A preliminary test of the validity of the sampling technique to provide uniform material for chemical analysis was made, and showed that, as regards the analytical data, the variation within samples was negligible in comparison with that between treatments. The full results of the nitrogen analysis are given in Table III.

There is a definite progressive fall in the nitrogen percentage of all the fodders from series A to D. The mean values expressed as a percentage of series A demonstrate that this drop is particularly marked in the case of the series D plots of Uba. Therefore with this fodder, the high yields of herbage and dry matter from the six-months' cutting rotation coincide with a serious decrease in the nutritive value as determined from the crude protein percentage.

In order to eliminate seasonal variation and provide a valid comparison between the three grasses, the differences between corresponding readings were tabulated and an analysis of variance carried out on these difference data. The mean figures for all rotations are:

Elephant Grass	...	1·22 % nitrogen in dry matter
Guatemala Grass	...	0·92 % ,, ,,
Uba Cane ...	...	0·79 % ,, ,,

Table III. *Percentage nitrogen in dry matter (oven-dried sample).*

Series	Date of harvest 1933–4	Elephant Grass (E)	Uba Cane (U)	Guatemala Grass (G)
A	Sept. 25	1·54	1·21	1·30
	Nov. 9	1·49	1·40	1·32
	Dec. 25	1·94	1·31	1·29
	Feb. 8	1·78	1·45	1·38
	Mar. 26	1·68	1·00	1·21
	May 10	1·60	1·19	1·52
	June 25	1·43	0·89	1·29
	Aug. 9	1·71	1·28	1·50
	Mean	*1·65*	*1·22*	*1·35*
Percentage of series A		*100·00*	*100·00*	*100·00*
B	Nov. 9	0·95	0·68	0·92
	Feb. 8	1·06	0·86	0·78
	May 10	1·36	0·76	0·94
	Aug. 9	1·23	1·04	1·53
	Mean	*1·15*	*0·83*	*1·04*
Percentage of series A		*69·70*	*68·00*	*77·00*
C	Dec. 9	0·97	0·70	0·62
	Apr. 9	1·04	0·67	0·48
	Aug. 9	1·33	0·65	0·96
	Mean	*1·11*	*0·67*	*0·69*
Percentage of series A		*67·30*	*54·80*	*51·20*
D	Feb. 8	0·78	0·36	0·53
	Aug. 9	1·19	0·56	0·69
	Mean	*0·98*	*0·46*	*0·61*
Percentage of series A		*59·50*	*37·70*	*45·30*

On a probability of 0·02, a difference between these means greater than 0·10 is significant, proving that the Elephant Grass is richest in nitrogen content and the Uba Cane poorest. The interaction variance was non-significant. This indicates that on the average the grasses have responded in a similar manner to changes in the cutting rotation, the relative differences in nutritive value being maintained through all four cutting rotations. The superiority of the Elephant Grass may have been accentuated by the fungal epidemic, which retarded growth and resulted in the production of a less mature type of herbage at each harvest.

By combining Tables II and III showing the dry-matter yields and nitrogen percentages respectively, it has been possible to calculate the total yield of crude protein for each series and each harvest as recorded below.

For each of the fodders, the maximum yield of crude protein is produced from the series B plots in which a three-month cutting rotation has been used. This contrasts with the progressive rise in total yield of

Table IV. *Yield of protein in lb. per acre.*

Date of harvest	Series A			Series B			Series C			Series D		
	E	G	U	E	G	U	E	G	U	E	G	U
Sept. 25	218	156	166	—	—	—	—	—	—	—	—	—
Nov. 9	102	123	92	353	303	342	—	—	—	—	—	—
Dec. 9	—	—	—	—	—	—	461	260	478	—	—	—
Dec. 25	51	129	75	—	—	—	—	—	—	—	—	—
Feb. 8	26	66	50	181	164	94	—	—	—	295	407	430
Mar. 26	18	57	54	—	—	—	—	—	—	—	—	—
Apr. 9	—	—	—	—	—	—	91	91	83	—	—	—
May 10	10	33	32	37	105	92	—	—	—	—	—	—
June 25	14	48	33	—	—	—	—	—	—	—	—	—
Aug. 9	13	68	33	33	232	167	22	176	118	27	177	198
Totals	452	680	535	604	804	695	574	527	679	322	584	628

green herbage and dry matter from series A to D (*vide* Figs. 2 and 3). It proves that, with these three fodders, the increased yield of roughage from cutting rotations exceeding three months is more than offset by the loss in protein percentage. Not only will the total yield of crude protein be less from the C and D series, but the herbage itself will have a much higher crude-fibre percentage (1), and in consequence the digestibility coefficient is likely to be smaller. It would appear, therefore, that in order to obtain the maximum nutritive value per acre a cutting rotation of approximately three months should be adopted. On this rotation, the Guatemala Grass is the best of the three fodders.

An average sample of Guatemala Grass from series B gave the following analysis:

	Percentage
Moisture	76·78
Crude protein	1·52
Crude fibre	7·33
Ash	2·18
Carbohydrate + ether extract	12·19
	100·00

The composition of the dry matter is therefore somewhat similar to that of a rather low-grade sample of meadow hay (20). The Guatemala Grass herbage from a three-month cutting rotation has probably a wider nutritive ratio but a higher percentage of soluble carbohydrates.

VIII. FIELD OBSERVATIONS.

Modern statistical methods have not only revolutionised the technique of agricultural experimentation, but have also greatly added to the accuracy of the ultimate conclusions. As a consequence, there is to-day rather a tendency to over-stress the importance of yield and similar data and to forget that there are other factors which may not permit of statistical interpretation but are of considerable significance in the final

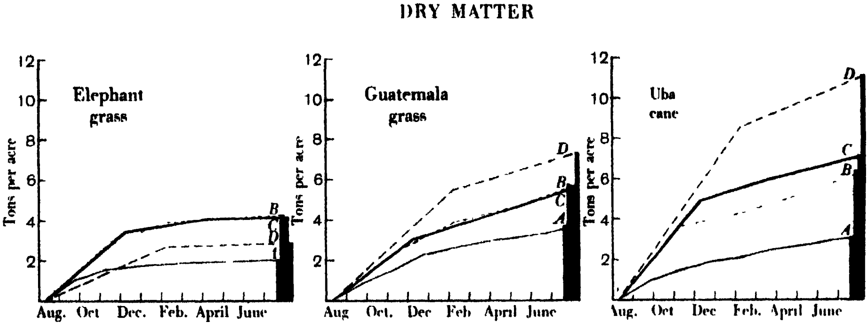

Fig. 2. Showing the relative yields of dry matter from the different fodders and cutting series during the experimental period.

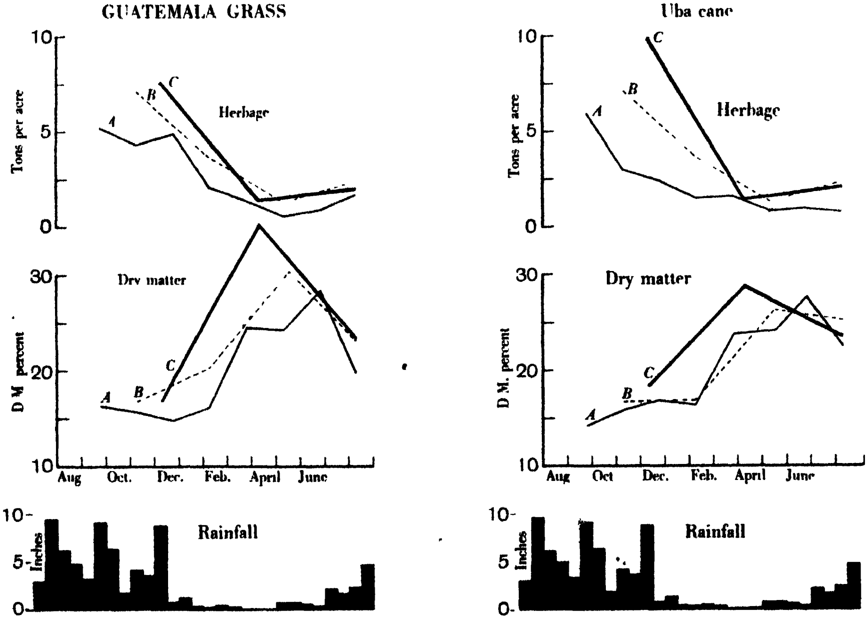

Fig. 3. Comparing the dry-matter percentages and 45-day increments with the rainfall.

summing up of results. In order to ensure that, in the present experiment, all possible sources of information were tapped, a careful examination of every plot was made at periodic intervals, and full notes kept regarding the comparative conditions and progress of the fodder grasses under the various treatments. These field notes have provided no inconsiderable amount of supplementary information, a summary of which is appended.

(*a*) *Weed control.* It is necessary with every perennial fodder crop to take into consideration not only the potential nutritive value per acre per annum but also the amount of cultivation required to maintain this yield level over a reasonable period of years. The ideal soilage crop is the one which is easy to establish and which, once established, will look after itself without further cultural treatment and maintain a pure stand under any reasonable mowing rotation. In an insular tropical climate like that of Trinidad, it is rare that the climatic conditions are so rigorous as to produce any great mortality in a healthy perennial fodder crop. If it is assumed that the fertility of the soil is maintained by occasional dressings of some appropriate manurial mixture, the chief factor that the crops will have to contend with is weed competition. The variety of weeds that may make their appearance in arable land in the tropics is legion, and many of them are of a particularly obnoxious and persistent nature. The struggle to keep the land even moderately clean never ceases: even in the drier seasons of the year, when weed growth decreases, the crop plants also are less productive and are slower in developing the necessary ground cover to eradicate extraneous vegetation. Hence, the ability a cultivated fodder has of effectively smothering weed growth is of great importance in assessing its value to the live-stock farmer. The more frequently the crop is cut, the greater the opportunity the weeds have of establishing themselves in the interstool spaces. This was demonstrated clearly in the experiment. In all the grasses, series A produced a very thick crop of weeds, while the land in series D was practically free from them, in spite of the fact that in the latter case the weeds were mown only twice in the twelve months, as against the eight harvests in series A. Series B and C were reasonably free of weed growth, but the land was by no means as clean as in series D.

There was also a very decided correlation between the variety of grass and the quantity of weeds. The Elephant Grass was undoubtedly the dirtiest due once again to the fungal epidemic which by the second half of the experimental period had killed off some of the stools and considerably reduced the productivity of the remainder. Guatemala Grass is very much superior in weed eradication to the Uba Cane. The difference is apparent in all four cutting rotations but most marked in series A and B. In series A, the plots of Uba Cane at the end of the experiment were completely overrun with weeds: the plots of Guatemala were weedy, but the crop appeared to be more than holding its own. In series B the weeds were moderately thick in the Uba Cane, but the corresponding plots of Guatemala could be described as reasonably clean. The explanation of this superiority of Guatemala Grass lies firstly in its ability to give a quicker smother after each harvest, as, on the average, it ratoons more rapidly than the Uba. Furthermore, the Guatemala Grass has a more superficial root system with a much greater lateral spread, which, with the 3 × 1½ ft. spacing adopted, gives a fine network of roots all through the first 6 in. of soil, making it very difficult for weeds in an immature condition to survive. The Uba Cane has a deeper more compact type of root development, which leaves unoccupied sections of soil between the stools where weeds get effectively established. This efficiency of Guatemala Grass as a weed eradicator has been demonstrated in general farm practice. There are records of good fields of this grass still surviving after being used for soiling for over five years without cultivation.

(*b*) *Regeneration.* There are two distinct ways in which the grasses can give rise to the ratoon crop, either by terminal or bud development. In the former, the original growing point of each tiller is not cut at harvest, but remains on the stump and by continued development produces the new foliage. The first few leaves are, in consequence, truncated, as the tips have been removed for fodder, but as there is no check to growth, ratooning is rapid and a good ground cover is quickly established. In bud sprouting, the growing tip of each leaf is removed with the rest of the herbage at harvest time, and the new crop develops from the dormant buds at the base of the old tillers. Under this system, there is a lag of a few days between cutting and visible signs of sprouting, and ratooning is consequently slower. On the other hand, it frequently happens that on any one tiller, several buds sprout simultaneously, and the number of tillers ultimately produced on the stool is increased. It was noticed that the particular cutting rotation used had a marked influence on the method of regeneration adopted by the plant, terminal development, in general, being associated with frequent harvests and bud development with a more lenient cropping. From the practical aspect, there is little doubt that a combination of both types of regeneration is desirable, terminal to avoid an excessive growth check and give an early ground cover against weeds, and bud sprouting to augment the number of tillers per stool and increase the yield of succulent herbage at harvest time. This ideal was attained in the case of the series B plots, in which, after each harvest, both systems of regeneration occurred in about equal proportions.

(*c*) *Hardiness*. The prolonged dry spell during the first half of 1934 provided an excellent test of the reactions of the fodders to semi-drought conditions. The Elephant Grass is a variety that thrives best when soil and atmospheric humidity is high, even to the extent of swamp conditions. Guatemala Grass is rather similar to the Elephant Grass in this direction, but to a less degree. Uba Cane, on the other hand, is not a crop that successfully withstands waterlogging; with Uba, heavy yields in high rainfall areas are only obtained in soils that are well drained. It is deeper rooted, and appeared to be less affected by the drought than the other two varieties. The Elephant Grass was most severely hit by the dry season, partly because its general vigour had already been undermined by the fungal infection. The herbage of the Guatemala Grass changed its character, the leaves becoming dry and yellowish in appearance and rolled longitudinally into narrow spills. In comparison the Uba looked greener and more succulent and more palatable. Recently mown plots of all species seemed to suffer less from the drought than those bearing older herbage. This is presumably due to the much larger total leaf surface on plots covered with a luxuriant growth of semi-mature foliage and the inability of the root system to cope with the transpiration currents. In consequence, as a field practice it might be advisable to shorten the cutting rotation in the dry season. There was no evidence of any stool mortality on account of the drought, and although the reactions of the three fodders to dry season conditions are different, they are all certainly drought hardy when grown in Trinidad.

Perennial soilage crops should rarely be allowed to reach the flowering or seeding stage. When this occurs, there tends to be a transference of the nutritive elements in the vegetative portions of the plant to the reproductive, new foliage development is retarded, and the existing herbage often becomes more fibrous, less digestible and less palatable. With the three fodders tested, there appears to be little likelihood of flower production in any quantity. The season was peculiarly favourable to flower development, yet the records show that only a few isolated inflorescences made their appearance on the Elephant and Guatemala Grass, and none on the Uba Cane. This statement, of course, refers to these crops utilised for soiling with a maximum period between harvests of six months. That even this limited amount of flowering is exceptional is evidenced by the local farmers who assert that they had not previously seen flowers on any of these grasses when grown for fodder production.

(*d*) *Root development*. It has been noted by other workers (7, 8) that too frequent mowing not only reduces herbage productivity but results in a poorer root development and ultimately in a smaller, less virile type of stool. An attempt was made to prove this for these tropical fodders by digging up a number of stools selected at random from the various treatment series, washing the soil from the roots and then drying and weighing them. An arbitrary comparison of the root systems thus exposed revealed that the larger ones were in general associated with the longer cutting rotations. Similarly, tiller counts taken after the last harvest in August 1934 showed that the greatest number of tillers and presumably therefore the largest stools occurred on plots having a long cutting rotation. In both instances, due to variability in soil, sprouting habit, etc., the data collected were too variable to permit of statistical proof of these facts. Differences as judged by eye were quite distinct, and in the light of similar findings on other research stations, it may be assumed that the general rule of improved stool virility with the longer cutting rotations does apply.

(*e*) *Propagation.* Tropical fodder grasses are normally propagated by root division. Two to three tiller root sections are preferable to singles, as they ensure a higher percentage of success and promote quicker development and an early ground cover. The Elephant Grass was definitely superior to the other two species as regards rapidity of establishment. In Trinidad, planting should be carried out shortly after the rainy season has started, from May to July. As the fodders grow most luxuriantly during the wet months, this time of planting facilitates successful propagation and accelerates the production of large healthy stools during the initial period. As with all perennials, this is a critical stage and may determine the ultimate yield potentiality of the crop. The planting distance of 3 × 1½ ft. proved satisfactory, as it permitted intercultivations during the first four to six months, and yet left the established crop close enough to inhibit weed growth on an average cutting rotation.

IX. SEASONAL EFFECTS.

It has been pointed out (1) that the climatic factor in the insular tropics which has most influence on crop growth is the rainfall incidence. The previous experiment (1) was carried out over a twelve-month period in which the rain distribution was exceptionally even. This fact, combined with the limited data available, made it impossible to show any decided correlation between the monthly precipitation and the yield of fodder. The present experiment, on the other hand, happened to coincide with a prolonged dry-weather spell, and, in consequence, herbage yields are closely correlated with the monthly rainfall (*vide* Fig. 3). As a result of the fungal attack, the growth of the Elephant Grass was not normal, and the data from this species have been omitted in demonstrating these correlations. With most crops there is a lag between the rain incidence and the response in the plant. Allowing for a latent period of one week, the total number of inches of rain during the appropriate 45 days preceding each harvest of series A has been recorded, and the correlation between these values and the yield of herbage in series A has been worked out for Guatemala Grass and Uba Cane. The correlation coefficients are:

Guatemala Grass, $r = +0{\cdot}987$ with a probability of significance less than 0·01.

Uba Cane, $r = +0{\cdot}802$ with a probability of significance less than 0·02.

In a normal year, similar correlations would presumably be present, but the difference in yield between the dry and wet months would not be so great and the plot variation might make them difficult of proof. The appended data give an idea of the way in which the experimental period deviated from the average and explains why the total herbage yields are subnormal. There is reason to believe that unfavourable conditions such as drought tend to exaggerate the disparity in yield between a severe and a lenient cutting rotation (7). Therefore the prolonged dry spell may have accentuated the differences between treatment series A, B, C and D, and may be partly responsible for results which are satisfactorily conclusive.

Rainfall in inches at the Imperial College of Tropical Agriculture, Trinidad.

Month	Mean for five-year period, 1928–33		Experimental year, 1933–4	
Aug.	8·47		11·16	
Sept.	6·30		12·33	
Oct.	4·92		8·22	
Nov.	8·66		7·68	
Dec.	5·88		9·51	
Jan.	3·17	34·32	1·73	9·55
Feb.	1·20		0·83	
Mar.	1·85		0·47	
Apr.	1·92		0·16	
May	5·49		1·48	
June	10·07		1·08	
July	10·62		3·80	
Total	68·55		58·45	

While there is considerable difference between the totals, the extent of the drought is best demonstrated by comparing the number of inches falling during the seven months January to July. In 1933–4 the precipitation was only 28 per cent. of the normal as estimated from five years' data.

In the herbage graphs in Fig. 3, the increments are taken as being proportional to the yield during the preceding 45 days. In plotting these graphs, the periodic yields of series B have been divided by 2, and of series C by $\frac{8}{3}$ in order to reduce the increments in these series to the same basis as those in A. The series A graph, being plotted from eight readings, shows most clearly the correlation between yield and rainfall. It is apparently most marked in the case of the shallow-rooted Guatemala Grass, which responds at once to any increase in the number of inches of rain. The difference between the correlation coefficients for Uba and Guatemala Grass expressed in terms of Z, is 1·4014, with a standard error of ± 0·8164. The difference is therefore not quite statistically significant. Series B and C also show a decided drop in yield with the coming of the dry spell. It is interesting to note that the yield drop is most acute in the longer cutting rotations, the growth rate in series C falling down to about the level of series A during the dry weather. This provides further evidence that, in the dry season it is better to cut the fodder relatively frequently and avoid the development of semi-mature foliage which suffers most from drought. Furthermore, other research workers (9) have noted that the conservation of moisture in relation to the production of phosphorus and nitrogen was greatest in a frequent cutting system. From various aspects, therefore, a reduction in number of days between harvests in the dry season is indicated.

The dry-matter percentage graphs demonstrate a progressive decrease in moisture content from series A to C, the difference being accentuated during the low rainfall periods, and most marked in the case of the Guatemala Grass. In all series there appears to be a negative correlation between the dry-matter percentage and the number of inches of rain. The correlation coefficients for series A have been calculated and gave the following values:

Guatemala Grass, $r = -0{\cdot}747$ with a probability of significance less than 0·05.

Uba Cane, $r = -0{\cdot}792$ with a probability of significance less than 0·02.

It follows, therefore, that the drop in yield of dry matter during periods of low rainfall is not proportionately as great as the decrease in the quantity of herbage.

In (1), a marked negative correlation between protein percentage and rainfall was noted. In periods of high productivity the protein percentage falls. A similar negative correlation is not obtained if the data from the present experiment are examined. The reason for this apparent reversal of the previous year's results is that the second season was abnormally dry, giving rise to drought conditions as contrasted with the usual dry weather ones, in which the soil moisture is never unduly depleted. It is an accepted fact that coincident with drought, a marked fall in the protein percentage of fodder crops generally occurs, and that is what has happened here. Thus, in seasons of heavy rainfall and high productivity the protein percentage will be relatively low; with a decrease in productivity associated with periods of reduced rainfall a rise in protein content may be anticipated. If, however, the decrease in precipitation is so marked as to develop semi-drought conditions, a reduction in the protein constituent will again become inevitable.

X. COMPARISON OF FODDER RESEARCH UNDER TROPICAL AND TEMPERATE CONDITIONS.

There is no doubt that the laws governing pasture and hay crop growth in the temperate zone apply in general to the cultivated fodder grasses of the insular tropics. Here, as in the United Kingdom, a lenient cutting rotation favours increased yields in bulk, herbage and dry matter, and as a result of the greater maturity of the crop at harvest, causes a drop in the relative, and ultimately in the absolute, protein and mineral yields, and provides a type of fodder with a much higher crude-fibre percentage and greatly reduced digestibility. Too frequent cuttings inhibit proper development of the plant, and produce non-virile individuals with a poor root system and little foliage output. A fall in productivity and a rise in the dry-matter, crude-protein and ash percentages are associated with periods of low rainfall. A slight alteration in the height at which the crop is cut is sufficient to cause a considerable modification in the growth habit and in the gross yield (1, 5, 7, 9).

There are certain aspects along which tropical conditions show considerable contrast with those in temperate regions. The most obvious one is that, in the humid tropics, growth is continuous throughout the year and perennial crops have no winter rest period. As a direct result of this the total fodder production is very much greater, with a possible maximum approaching 20 tons dry matter per acre per annum; 20 per cent. of this would be considered a satisfactory yield from a hay + aftermath crop in England. In the tropics, also, the wastage of plant foods by leaching is normally much greater, so that it is advisable, if a high level of productivity is to be maintained, to apply periodic dressings of manure. It has been shown (18) that, even in temperate regions where both yields and losses of plant food from the soil are less, there is a progressive drop in the annual tonnage of bulk produce obtained from a hay crop grown in the same field for five years in succession. This is attributed, after making allowance for seasonal and age differences, to the general depletion of soil fertility as a result of continuous cropping without manuring.

Much of the recent research in Europe has been devoted to pasture improvement and grazing technique. The artificial establishment of semi-permanent close-grazed cultivated pastures in the wet equatorial zone is practically unknown. The maintenance of the species sown in the seed mixture would certainly be uneconomical and in most cases impracticable. Pasturage is therefore limited to grazing on the natural herbage found in suitable localities, and the cultural treatment confined to an occasional uprooting of certain poisonous or otherwise undesirable intruders in the plant population. Most of the species found in such natural pastures are hardy perennials of low nutritive value, a typical example being Savannah Grass (*Axonopus compressus*). Overstocking is also a common evil and the annual yield of nutriment is consequently unnecessarily low. Pasturing on scientific lines is rarely practised. Except on waste lands of very low fertility, the establishment of long-lived hardy perennial cultivated fodders is undoubtedly the most economical method of utilising the soil.

These fodders are generally used as soilage crops, but recent experiments indicate that they will continue to thrive under a controlled system of rotational grazing, a practice which has some obvious advantages (19). In either case, storage facilities are not required, and in comparison with natural pasturage, the small cost of cultivation and utilisation is more than offset by the increased head of stock that can be maintained. Thus, until information regarding the growth and nutritive quality of the more important perennial fodders has been augmented, experiments with these crops should take preference, in the wet tropics, over pasture research.

Stapledon and Davies (18) point out that in England, "the seasonal productivity in pastures is affected as much or more by the plant's functional periodicity as by meteorological conditions". The spring flush in all seasons produces relatively rapid vegetative growth, leading to the summer flowering and seeding period and ending in the gradual dying down of active growth in the autumn. The data indicate that as much as 70 per cent. of the total yield may be produced in the two months, May and June. In the insular tropics, the similarity in climatic conditions throughout the year as regards temperature, sunshine, length of day, etc., induces a growth habit in the plant which shows much less variation from month to month. Any marked alteration in productivity is directly traceable to a change in the soil-moisture content, high yields following closely on periods of heavy rainfall. Soil and atmospheric temperatures, number of hours sunshine, and humidity, except as secondary effects of dry weather conditions, are of little count. There is, in consequence, a closer correlation between the periodic productivity and the rainfall distribution over the year, and associated with this variation in the yield from month to month are corresponding changes in composition and nutritive value.

Soilage crops in the tropics are essentially of the type of hardy perennial grasses, producing an abundant growth of a somewhat coarse roughage. They are, in general, inferior in total nutrient percentage and especially in protein and minerals (11–16) to the forage crops of the temperate zone. The difference in quality need not be as great as was originally anticipated. By choosing a suitable species and adopting a correct mowing rotation, a fodder that in dry-matter composition is not greatly inferior to average meadow hay can be obtained. Furthermore, this inferiority in nutrient composition in the tropical produce is in part at least compensated for by the higher digestibility percentage that is normally obtained from tropical stock when compared with their prototypes in Europe. It is not possible by cutting the produce at a very immature stage, to obtain a watered concentrate such as is shown to be practicable from certain pastures in England. These general differences in composition between the fodders of the tropical and temperate zones are in part attributable to the higher proportion of leguminous species in the pasture population of cooler countries. Richardson (17) is responsible for another tentative explanation of the phenomenon. His theory is that there is a differential effect of climate on the rate of mineral uptake on the one hand and on the rate of growth on the other, tending to produce a higher carbon-nitrogen ratio in the tropics.

Results up to the present indicate that with tropical soilage crops a relatively long interval between harvests is advisable in order to attain the optimum conditions for yield, quality and general virility. For example, a three-month cutting rotation has been suggested for Guatemala Grass as compared with the maximum yields of nutriment obtained from an English hay mown every 4–5 weeks. It is possible that with the limited active growing period of the temperate world, fodder crops are more able to withstand relatively severe cropping. They have in every year a five to six months' dormant period as contrasted with the continuous growth and repeated ratooning of the cultivated fodders of the tropics.

XI. FUTURE RESEARCH.

In this experiment, the Guatemala Grass has successfully demonstrated its superiority as a soilage crop. Only three species of fodder were tested and there are many others which might equally well have been selected for trial. Observation plots of six of these alternative fodders indicate that at least two of them show particular promise, namely, Para Grass (*Panicum barbinode*), Coimbatore Cane (*Saccharum sinense* var. *Co. 213*). In consequence, an experiment designed to compare these with the Guatemala Grass has subsequently been started. Due to its disease susceptibility, the Elephant Grass has proved a failure in Trinidad. It is, however, one of the most popular tropical fodders of to-day, and has recently been introduced into many new localities with marked success. There appears to be a possibility of isolating a strain of this grass which is resistant to the *Helminthosporium* sp., and experimental work in this direction has also been inaugurated.

The comparison of these fodders has of necessity been limited almost wholly to the agricultural aspects of the problem. The final ratification of the tentative theories advanced lies in the province of the chemist. Fagan and Jones (7) point out that in order to assess the intrinsic value of any fodder, an accurate knowledge of at least four factors is required, namely, yield, persistency, composition and digestibility. Up to the present, in this fodder grass research, data for the first three of these are available. Due to lack of facilities, it has not yet been possible to carry out any digestibility trials. It is recognised that chemical analyses alone provide only a rough guide to the relative nutritive value of any ration. Kirsch (10) definitely states that the correlation between crude fibre and feeding value is not of an order that permits of the omission of digestibility trials. Until these become feasible, it has been considered advisable, in this experiment, to limit the analytical work to the dry-matter and protein determinations which in themselves can be used as indicators of the different grades of produce. More detailed analysis would mean considerable extra labour without providing a much more accurate estimate of the nutritional values. One aspect that it may be possible to tackle in the near future is the mineral content. There is reason to believe that in young produce grown on the College soils which are manured, this is fairly satisfactory (1). That a mineral deficiency in other localities may be anticipated is shown by a report (11) from British Guiana in which seven different pasture species were tested and found to have an inadequate mineral percentage for live-stock nutrition. It is generally admitted too that the mineral percentage of pastures grown in tropical and subtropical areas is lower than that recorded for the temperate zone (12–15). The fluctuation in the mineral composition of the important tropical fodders as a result of differences in soil, season, species and maturity of herbage is, in itself, an intricate problem demanding study in considerable detail at an early date. In conjunction with this, complete analysis of the various types of produce at different stages of development are required and determinations of digestibility coefficients and starch equivalent values are essential if accurate rationing is to be achieved.

XII. SUMMARY.

The experiment was designed to compare under Trinidad conditions three varieties of fodder grass and ascertain the correct stage at which each should be cut to give the maximum nutritive value per acre. It consisted of a yield trial in the form of a Latin square in which the treatment series included four cutting rotations on main plots and three varieties of fodder grass on subplots as appended:

Main plot treatments:
Series A = plots harvested every 45 days
,, B = ,, ,, 90 ,,
,, C = ,, ,, 120 ,,
,, D = ,, ,, 180 ,,

Subplot treatments:
Series E = Elephant Grass.
,, U = Uba Cane.
,, G = Guatemala Grass.

At each harvest representative samples of the produce from the various treatment series were collected for chemical analysis (IV).

The data proved that, on the average, for either herbage or dry matter, the longer the cutting rotation up to six months the greater the yields tended to be. As regards those yields also the varieties showed distinct differences and can be arranged:

(1) Uba Cane
(2) Guatemala Grass } in the proportions 100 : 81·2 : 48·3 per cent.
(3) Elephant Grass

The low yield of Elephant Grass is attributed to a severe attack of a fungal parasite—*Helminthosporium* sp.—on the foliage.

It was shown that, for each variety individually, cutting as often as every 45 days (series A) decreased productivity, while the six-month cutting rotation (series D) gave with Uba and Guatemala a significant increase, but with Elephant Grass a significant decrease in total yield when compared with the data from the plots of the B and C series (III and V).

Height measurements taken at every harvest proved that the height of the herbage when it is cut is positively correlated with the yield and the cutting rotation, the greater the yield and the longer the cutting rotation, the taller the grass (VI).

In chemical composition, the Elephant Grass is some 2–3 per cent. lower in dry-matter content than the other two fodders. In all the varieties there is a progressive rise in dry-matter percentage and a progressive fall in protein percentage from series A to D. Of the three grasses, the Elephant Grass is significantly better and the Uba Cane significantly poorer in protein than the Guatemala Grass. For each of them the maximum nutritive value per acre, as estimated from the total protein yield, is obtained from series B in which a three-month cutting rotation was adopted. This contrasts with the high yield of bulk herbage from the series D plots of Uba and Guatemala, and indicates that, with too mature herbage, the loss in total digestible nutrient will more than offset any increase in productivity. Of the three fodders, the Guatemala Grass gives much the highest yield of protein per acre (VII).

Field observations demonstrated that sprouting of the ratoons can occur in two ways, namely, by bud, and/or terminal development of the tillers. The particular method which will predominate depends primarily on the length of the cutting rotation, and it was pointed out that simultaneous development by both methods is desirable to produce a quick cover and a dense herbage. With Guatemala Grass this occurs on the optimum cutting rotation of three months. As regards weed growth, the longer the cutting rotation the cleaner the land. Guatemala Grass was the most efficient and Elephant Grass the least efficient weed eradicator. Even with a six-month period between harvests, there appeared to be little danger of premature flowering in any of the three grasses. The deep-rooted Uba Cane suffered least during a prolonged period of drought, but all three varieties showed themselves to be quite capable of successfully withstanding abnormally dry weather conditions. Only one serious pest made its appearance, namely the *Helminthosporium* sp. on the Elephant Grass. The infestation was very severe and makes it inadvisable to grow this particular fodder in localities where the virulent form of the fungus is endemic. Root and tiller examinations revealed that the short cutting rotations are associated with smaller less vigorous stools and decreased root development (VIII).

As regards seasonal variation, a marked positive correlation between rainfall and productivity was noted, and a negative one between rainfall and dry-matter percentage. The negative correlation recorded in the original experiment with Elephant Grass between the number of inches of rain and the protein percentage was not present. The absence of this anticipated correlation is apparently due to the prolonged period of drought (IX).

With reference to fodder crops, tropical conditions are compared and contrasted with those prevailing in the temperate zone. The theory that, in the humid tropics, pasture experiments should take second place to research on perennial cultivated fodder grasses is advanced (X).

ACKNOWLEDGMENTS.

The writer has great pleasure in acknowledging his indebtedness to Prof. R. Cecil Wood for providing facilities for the research and for his continued interest in the progress of the work. He wishes to record his sincere thanks to the staff of the Chemistry Department of the Imperial College—Prof. Hardy, Mr Duthie and Mr Rodriguez—for their willing co-operation in carrying out the chemical analyses. Without the analytical data, the utility of the experiment would have been greatly reduced.

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(*Received April* 15*th*, 1935.)

APPENDIX.

Yields of green herbage in lb. per subplot.

	Series A												Series B											
Main plot No. ...	2			8			11			13			1			7			12			14		
Date of harvest	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U
Sept. 25	305	232	394	267	213	300	270	187	167	358	191	232	—	—	—	—	—	—	—	—	—	—	—	—
Nov. 9	129	163	101	160	181	132	135	158	106	173	167	124	583	708	1086	417	617	857	512	587	703	436	341	749
Dec. 9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dec. 25	27	159	103	96	209	101	65	214	99	55	183	77	—	—	—	—	—	—	—	—	—	—	—	—
Feb. 8	15	76	53	32	89	61	22	88	66	26	84	52	254	291	164	168	227	156	317	373	232	208	267	179
Mar. 26	7	44	62	15	63	66	12	49	70	17	60	59	—	—	—	—	—	—	—	—	—	—	—	—
Apr. 9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
May 10	5	22	37	5	25	27	11	27	35	14	27	26	24	101	141	31	98	110	11	121	164	44	92	101
June 25	9	29	45	4	25	17	18	49	47	18	45	41	—	—	—	—	—	—	—	—	—	—	—	—
Aug. 9	10	47	40	3	43	8	14	100	42	22	70	40	13	191	208	47	199	143	7	165	213	64	182	151
Totals	*507*	*772*	*835*	*582*	*848*	*712*	*547*	*872*	*632*	*683*	*827*	*651*	*874*	*1291*	*1599*	*663*	*1141*	*1266*	*847*	*1246*	*1312*	*752*	*882*	*1180*

	Series C												Series D											
Main plot No. ...	3			6			9			16			4			5			10			15		
Date of harvest	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U	E	G	U
Sept. 25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nov. 9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dec. 9	432	690	1051	421	915	1284	811	707	714	546	480	1087	—	—	—	—	—	—	—	—	—	—	—	—
Dec. 25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Feb. 8	—	—	—	—	—	—	—	—	—	—	—	—	478	1060	1504	456	951	1359	395	978	1231	502	807	1384
Mar. 26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Apr. 9	70	116	104	58	162	113	65	176	120	124	147	144	—	—	—	—	—	—	—	—	—	—	—	—
May 10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
June 25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aug. 9	31	154	141	22	216	298	18	271	228	8	242	201	8	400	398	13	288	460	25	331	299	74	362	639
Totals	*533*	*960*	*1296*	*501*	*1293*	*1695*	*894*	*1154*	*1062*	*678*	*869*	*1432*	*486*	*1460*	*1902*	*469*	*1239*	*1819*	*420*	*1309*	*1530*	*576*	*1169*	*2023*

Treatment totals

	Series A	Series B	Series C	Series D	*Variety totals*
Elephant Grass	2319	3,136	2,606	1,951	*10,012*
Guatemala Grass	3319	4,560	4,276	5,177	*17,332*
Uba Cane	2830	5,357	5,485	7,274	*20,946*
					Grand total
Series totals	*8468*	*13,053*	*12,367*	*14,402*	*48,290*
	48,290				

Index.

Series A. Harvested every 45 days
,, B. ,, 90 ,,
,, C. ,, 120 ,,
,, D. ,, 180 ,,

E = Elephant Grass, G = Guatemala Grass, U = Uba Cane.

THE RETENTION OF PROTEIN BY GROWING PIGS.

By J. H. W. Th. REIMERS and L. H. BARTEL.

(Department of Animal Husbandry, Stellenbosch-Elsenburg College of Agriculture of the University of Stellenbosch, South Africa.)

INTRODUCTION.

GROWTH in young animals is an inherent quality possessed by them to multiply the cells of the different organs and to develop these organs in proportion to each other, resulting ultimately in individuals which in conformation, constitution and function are the like of the mature animal of the species and breed to which the young belongs.

All these changes and increases of matter and function in the animal body from birth till maturity can only be brought about when the necessary material to affect or to support these changes can be collected or metabolised from the food given to the animal, and when the surrounding conditions do not prevent or retard the normal development which we usually call "growth".

In feeding experiments with growing animals we must therefore aim at the determination of the minimum, optimum and maximum amounts of certain constituents of the food of great importance for normal growth.

Experiments are reported here in which an attempt has been made to determine these amounts for the protein of the food.

LITERATURE.

Very few researches on the retention of protein and the total energy required to promote normal growth in pigs have been carried out, and not many nutrition balances showing this can be found. There are, however, on record quite a number of practical experiments dealing with the feeding of young pigs from which figures may be derived as to the necessary amounts of protein and energy for growing pigs.

We present these figures shortly in Table I. Bracketed figures are calculated from the experimental data by the authors.

Extensive researches on the digestibility and retention of protein,

Table I.

Author	Average increase in live weight per day in gm.	Total g. of digested protein per day	Total S.E. per day	Live weights of animals in kg.	Average increase in live weight per day in gm.	% retention of protein digested	Total amount of protein necessary per day in gm.	Total S.E. necessary per day in kg.
Nills Hansson (1)	—	—	—	40–50	—	—	180–220	1·3
Do.	—	—	—	50–60	—	—	200–240	1·6
Do.	—	—	—	60–70	—	—	205–250	1·8
Schmidt, Vogel and Zimmermann (2)	650	—	—	30–100	—	—	—	—
Chr: Wilkens (3)	500	—	—	34–64	—	—	(245–311)	(1·69–1·70)
Krüger (4)	617–681	—	—	? (40–70)	—	—	—	—
Lunde, Scharrer and Schropp (5)	562–620	—	—	36	—	—	—	—
Bünger (6)	—	—	—	51	645–860*	—	272–297	1·93–2·97
Do.	—	—	—	36	570–610*	—	238–251	1·18–1·61
Do.	—	—	—	36	591–539*	—	264–269	1·37–1·62
Nils Hansson, and Sven Bengtsson (7)	—	—	—	55–57	—	—	250–300	(1·61)
Do.	—	—	—	—	550–600	—	215–235	—
Do.	—	—	—	40	—	—	—	(1·331)
Do.	—	—	—	50	—	—	—	(1·61
Do.	—	—	—	60	—	—	—	(1·82)
Mitchell and Hamilton (9)	—	—	—	30–100	—	58	—	—
Mitchell and McClure (10)	—	—	—	50–55	—	±40	215–270	—
Do.	—	—	—	63–73	—	±42	262–294	—
Hjalmar Claussen (11)	—	—	—	30–40	—	—	200–225	1·26
Do.	—	—	—	40–50	—	—	225–255	1·61
Do.	—	—	—	50–60	—	—	255–275	1·86
Do.	—	—	—	60–70	—	—	275–300	2·07

* The animals have been fed *ad libitum*.

when various rations were fed to different breeds at different ages, have been carried out by Schmidt, von Schleinitz, Lagneau and Zimmermann (12), but the enormous number of valuable figures cannot be reproduced in this article through lack of space.

EXPERIMENTS I AND II.

To discover the influence the proportion of protein to energy may have on the retention of the protein and on the increase in weight of the animals, this first series of experiments was planned so that the animals received rather less total feed in a basal ration than prescribed in the standards for feeding. To this basal ration different amounts of skim milk, blood meal and barley meal were added to constitute rations for the four different groups in which the animals were divided. One group received a medium amount of protein and a medium amount of energy, the second group received about the same amount of protein and a low amount of energy, the third group received a larger amount of protein and a larger amount of energy, while the last group received a larger amount of protein but only a medium amount of energy.

Each group consisted of two barrows (Large Black × Tamworth). After feeding for three weeks, the ration was slightly altered in every group to meet the higher demands of the growing animal. In the experimental period of three weeks, every pig was kept during 48 hours in the middle of the week in a digestion box, its urine and faeces being carefully collected. During the week in which the change in food took place no digestion trials were made.

The faeces were mixed well and a sample was directly analysed for its nitrogen content. The urine, collected in large stoppered bottles, was measured and was preserved with a certain amount of sulphuric acid and chloroform, after which 5 c.c. was analysed for its nitrogen content.

One must presume that the excretion of faeces and urine during 48 hours corresponds with the intake during 48 hours, and that the total amount of retained protein is thus represented by the difference of the protein consumed during this time and the sum of the protein excreted in the faeces and in the urine. It is evident that errors can be introduced in this way, especially when the pigs do not regularly void their faeces or urine due to disturbing influences (restlessness in the piggery, differences in temperature and humidity of the air, slight digestion disturbances, etc.). It will always be difficult to evade these disturbances or to account for their influence, especially where the equipment for carrying out experiments of this nature is far from ideal.

After a short time the pigs settled down quite well and received rations consisting of a mixture of barley meal and pollard to which blood meal was added where rations with a high protein content were wanted. Each pig received also an additional supply of skim milk, and by varying the quantities of barley meal, pollard and skim milk and adding some blood meal in some cases, the differences in protein and energy of the rations could be easily established.

After a preliminary period of 7 days, the pigs of each group received for 2 weeks the same amount of the prescribed rations, while during the third week this amount was altered with only slight changes in the mixture to meet the demands of the growing animal. After the week in which the change took place the animals were kept on the new ration during the succeeding 4 weeks.

The amount of protein given to the pigs was calculated from the analysis of the feed; the amount of starch equivalents was calculated with the aid of Kellner's tables. After the digestibility of the protein had been determined from the nitrogen balance, the average ratio of digestible crude protein to total starch equivalents could be calculated.

Table II contains all particulars of the feeding during the first 3 weeks (periods I and II), while the rations given in the succeeding 4 weeks are those of periods III and VI in the same table.

The animals were all 134 days' old when the experiment started and weighed between 87 and 105 lb. (40–48 kg.).

Table II.

Period	Group	Total gm. of protein given per day	Total gm. of protein digested per day	Total S.E. consumed per day	Ratio digestible protein S.E.
I, II	I	196·8	163·5	0·805	1 : 4·92
I, II	II	175·3	143·9	0·596	1 : 4·14
I, II	III	271·5	211·4	1·111	1 : 5·26
I, II	IV	278·2	235·3	0·822	1 : 3·49
III–VI	I	206·6	171·0	0·982	1 : 5·74
III–VI	II	197·8	163·2	0·687	1 : 4·21
III–VI	III	325·9	246·5	1·466	1 : 5·94
III–VI	IV	290·7	237·2	1·010	1 : 4·26

To avoid individual differences as much as possible, the eight pigs used were all barrows, sired by the same father and from two mothers, who were full sisters. All animals were born on the same day, and were treated exactly alike prior to the commencement of the experiment. They had been running with the mothers on green lucerne and all got the same mixture of additional concentrates. The mothers, too, were treated alike during their time of pregnancy. Unfortunately one pig of group I had to be taken away at the beginning of the experiment on account of sickness. It was impossible to find another one to take its place at that time.

Digestibility of the crude protein of the rations.

In order to ascertain whether the rations were digested normally throughout the whole trial and to see whether any disturbances or

Table III.

Pig No.	Period	Total amount of protein consumed in gm.	Total amount of protein in faeces in gm. (N × 6·25)	Digested protein in gm.	Percentage digested
12	II	196·8	33·3	163·5	83·1
16	I	175·3	31·8	143·5	81·9
16	II	175·3	28·6	146·7	83·7
9	I	175·3	30·8	144·5	82·4
9	II	175·3	34·2	141·1	80·5
10	I	271·5	70·4	201·1	74·1
10	II	271·5	70·2	201·3	74·2
19	I	271·5	50·1	221·4	81·5
19	II	271·5	49·8	221·7	81·7
18	I	278·2	41·7	236·5	85·0
18	II	278·2	51·0	227·2	81·7
13	I	270·2	35·1	243·1	87·4
13	II	270·2	43·9	234·3	84·2
12	III	206·6	35·7	170·9	82·7
12	IV	206·6	38·3	168·3	81·5
12	V	206·6	40·2	166·4	80·5
12	VI	206·6	28·2	178·4	86·3
16	III	197·8	32·2	164·6	83·2
16	IV	197·8	40·0	157·8	79·8
16	V	197·8	50·4	147·4	74·5
16	VI	197·8	14·7	183·1	92·5
9	III	197·8	34·0	163·8	82·8
9	IV	197·8	30·7	167·1	84·5
9	V	197·8	33·8	164·1	82·9
9	VI	197·8	40·5	157·3	79·5
10	III	325·9	77·6	248·3	76·2
10	IV	325·9	85·4	240·5	73·8
10	V	325·9	93·2	232·7	71·4
10	VI	325·9	116·3	209·6	64·3
19	III	325·9	77·8	248·1	76·1
19	VI	325·9	59·6	266·3	81·7
19	V	325·9	68·8	257·1	78·9
19	VI	325·9	57·1	268·8	82·5
18	III	290·7	43·5	247·2	85·0
18	IV	290·7	42·9	247·8	85·2
18	V	290·7	77·2	212·5	73·1
18	VI	290·7	38·2	252·5	86·5
13	III	290·7	61·6	229·1	78·8
13	IV	290·7	65·8	224·9	77·4
13	V	290·7	47·2	243·5	83·8
13	VI	290·7	48·3	242·4	83·4

abnormalities in digestion occurred during any of the periods, the experiments detailed in Table III were carried out. They show the digestibility of the crude protein of the rations during the different periods when the faeces of the animals were collected.

As the true protein content of the rations was not determined, it must be remembered that whenever in this article mention is made of digested or digestible protein, *crude* protein is meant.

Comparing the digestibility of the protein during different periods of the same pig, it may be seen that the variations in digestibility of the same food by the same animal are small. Only in some cases greater differences are observed probably due to the short time (48 hours) each animal in each period remained in the digestion boxes. It was, for example, possible for an animal just before entering the box, to void faeces, and after having been in the box for 48 hours to void faeces again about half an hour after leaving the box. Part of these faeces might be derived from the 48 hours' food and would not be taken into account when determining the digestibility of that food, which showed in such a case to be a good deal better than where the voiding of faeces occurred just at the right time.

Examples may be found in the case of animal 16, period VI, in which the total amount of faeces and consequently the total amount of excreted protein were far below normal, and in the case of animal 10, period VI, in which the contrary happened.

To arrive at the digestibility of the food during the whole period, the average between the determined digestibility in the different digestion trials with the same ration was calculated and applied in the determination of the total amount of protein absorbed in the body.

Comparing whether there were important differences between both animals of the same group, it appears that in most cases these differences were negligible except in group III, in which animal 19 shows regularly a better digestion of protein than animal 10.

The differences between the average digestibility of the different rations are also not very important with the exception of the ration given to group III, which was less digestible than the other rations. This ration contains the maximum amount of protein and energy, and it may be that the large amounts of food consumed by the pigs were partly responsible for the decreased digestibility. The digestibility of the protein of all the rations is, however, quite normal, and differences in the results of the experiments certainly cannot be ascribed to abnormal differences in the suitability of the proteins of the food.

The retention of protein during the different periods.

The different amounts of nitrogen which were excreted in the urine of the animals during the digestion trials were determined in the same way. By multiplying the amounts of nitrogen with 6·25 the total corresponding amounts of protein responsible for the excretion of the nitrogen in the urine were calculated, and after subtracting these amounts from the absorbed protein the protein which was retained in the body during the trial could be arrived at.

From these figures the percentage retention of protein could be derived to afford a basis of comparison for the more or less nutritional and economical efficiency of the different rations. To control whether the increase in weight of the pig could be considered normal, each pig was weighed at the beginning of each week, the increase in weight per week providing a criterion for the growth of the animal.

From numerous practical experiments with a great number of pigs during the last ten years at this institution we know that an increase in weight of 450–550 gm. per day during the time the animals weigh between 40 and 65 kg. can be considered satisfactory, while from the time the animals weigh 65 kg. till they are about 100 kg., an average increase of 500–600 gm. per day can be considered normal. Higher increases have been obtained by feeding animals very high amounts of food in some experiments or by giving them food *ad libitum*, but these increases were obtained at such a higher cost of feeding that we did not consider it desirable in these experiments to try to establish these gains.

On the contrary, the rations were often rather a little below normal, which is shown by the rather low increase in weight some animals showed during the trial.

As the retention of protein will also be dependent on the total amounts of nitrogen-free nutrients given at the same time, and it was the intention to find at the same time standards for these nutrients too, the total starch equivalents of the rations are also given in Table IV together with the digested and retained protein.

Not to extend this table unduly the retentions in the different digestion trials are not given separately, but the average between the retentions during trials with the same rations have been given as well as the average digested protein during these trials.

All figures are expressed in grams per day, except the starch equivalents, which are always expressed in kilograms.

Discussing first the figures collected from each pig and comparing the pigs of the different groups with each other, pig 12 can form a good basis of comparison with the pigs of group IV, which received about the same amount of energy in their food as pig 12, but were given about 30 per cent. protein more.

Table IV.

Group	Pig	Period	Digested protein in gm.	Retained protein in gm.	Percentage retention	Total S.E. in kg.	Ratio protein S.E.	Increase in weight per day in gm.
I	12	II	163·5	97·1	59·4	0·805	1 : 4·9	194
II	16	I, II	145·1	52·0	35·8	0·596	1 : 4·1	340
II	9	I, II	142·8	48·1	33·6	0·596	1 : 4·2	226
III	10	I, II	201·2	112·7	56·0	1·111	1 : 5·5	616
III	19	I, II	221·5	157·9	71·1	1·111	1 : 5·0	372
IV	18	I, II	231·8	139·8	60·4	0·822	1 : 3·5	356
IV	13	I, II	233·7	179·5	76·5	0·822	1 : 3·5	389
I	12	III, IV, V, VI	171·0	122·7	71·8	0·982	1 : 5·7	324
II	16	III, IV, V, VI	163·5	82·2	50·3	0·687	1 : 4·2	186
II	9	III, IV, V, VI	163·0	87·9	53·9	0·687	1 : 4·2	194
III	10	III, IV, V, VI	232·8	134·9	57·9	1·466	1 : 6·2	445
III	19	III, IV, V, VI	260·1	179·4	69·0	1·466	1 : 5·6	656
IV	18	III, IV, V, VI	240·0	124·1	51·7	1·010	1 : 4·2	316
IV	13	III, IV, V, VI	235·6	177·0	75·1	1·010	1 : 4·3	324

Although the total energy of the ration of groups I and IV is about the same, the increase in live weight of pigs 18 and 13 is about double the increase in weight of pig 12.

The reason can only be sought in the increase in protein which enabled the animal to provide enough protein for the development of the various tissues.

That this explanation is very probable is also proved by the fact that the energy for the small increase of pig 12 was more than sufficient to prevent breaking down of protein for energy purposes, because had this happened the percentage retention of the protein would have been considerably lower.

We may conclude, comparing the two groups, that the energy was insufficient to obtain normal growth, and that the protein of group I was also insufficient for this purpose.

Group II, receiving less protein and less energy, shows nevertheless a higher increase in weight than pig 12 does during the first 3 weeks, although the increase is far below normal and is less than that of group IV. A very striking fact about this group is the conversion of protein into substances to provide energy, which results in a very low percentage retention of protein.

One gets the impression that the animals have collected as much nitrogen-free material as possible for an increase in weight, which must probably have consisted mainly of an increase in the fat of the body. How long these animals could carry on in this way will be discussed when comparing the figures of the periods III–VI.

The animals of group III received about as much protein as those of group IV, though pig 10 digested a little less. The daily increase of pig 10 is certainly very high, while pig 19 does not seem to utilise the high ration during the first 3 weeks as would be expected. The retention of protein ranges between 56 and 71 per cent., and shows that the amount of protein given was sufficient to support a good growth. The total amount of energy of about 1·1 S.E. seems quite adequate.

Three weeks cannot be considered sufficient to show marked differences in the effect of different rations, and the following periods have to be taken into account to arrive at more accurate conclusions. When the ration of pig 12 was increased in energy without a considerable increase in protein, the increase in weight was much improved and even the retention of protein reached a very high figure. This strengthens the aforementioned conclusion that the energy during the first 3 weeks was certainly insufficient, and even when brought up to nearly 1 S.E. it could not assume a good growth.

Group IV shows very markedly when compared with group I that it was not the insufficiency of the protein which alone prevented normal growth because increase in protein without increase in energy did not result in better gains. It may thus be stated that in this period 1 S.E. was insufficient.

Group II, which made gains of 340 and 226 gm. per day when fed a ration which was too low in energy, was compelled at the same time to break down protein, and to register a very low retention of this substance. When the total quantity of food per day was increased, it shows directly that the animals are chiefly engaged in filling up the shortage of protein in the growth made during the preceding periods. This results in a better retention, but because so much less is available for supplying energy, the total increase in weight is very small, being not more than 186 and 194 gm. per day. It may safely be stated that this group received a ration insufficient in energy and protein to enable normal growth to occur.

Group III does not receive much more protein than group IV, but is raised to a total consumption of 1·47 S.E. per day, and this has such a beneficial effect that pig 10, which showed during the first three periods an increase of 616 gm. in weight per day, proceeds to increase 445 gm., while pig 19, which did not clearly show any benefit from the high ration during the first 3 weeks, increasing only 372 gm., makes up for this by increasing 656 gm. per day in weight during the following periods.

This is certainly sound evidence on which to accept the conclusion that 200–220 gm. protein and 1·1 S.E. in the first 3 weeks and 230–260 gm. protein and 1·47 S.E. in the following 4 weeks can be considered normal rations for growing pigs.

The retention of protein is very variable, but as figures from insufficient rations do not represent normal conditions, the only retentions which can be used to derive conclusions from are those from group III, which vary from 56 to 71 per cent., pig 10 retaining 56 and 51·9 per cent., and pig 19 retaining 71·1 and 69 per cent. There is a very great individual difference between both animals, and here again an illustration may be found of the fact that although differences in digestion in pigs are usually very small, differences in the power of retaining certain nutrients may be considerable.

The experiments at the same time throw some light on the question of the retention of protein and fat in certain groups. While it is generally admitted that growth consists more in an increase of the nitrogenous matter of the body while fattening is recognised as the storing up of large amounts of fat in the body, it is not so generally known that the proportion of stored up fat and gained protein is far from being the same in growth.

Evidence of this fact can be found in a comparison of the figures of group III with those of group IV, both groups receiving about the same amount of protein but different amounts of energy and consequently increasing quite differently in live weight. While pig 10 increases 616 and 445 gm. in total of which 112·7 and 134·9 gm. are protein and pig 19 with a very great capacity for putting on protein increases 372 and 656 gm. totally with 157·9 and 179·4 gm. protein, the pigs of group IV, which do not increase more than 316–389 gm. per day, increase in protein as follows: pig 18 by 356 and 316 gm. total increase, gains 139·8 and 124·1 gm. in protein, pig 13 by 389 and 324 gm. increase in weight gains at the same time 179·5 and 177 gm. protein per day. It is apparent that the proportion of protein increase to fat increase is in group IV far more favourable for the protein than in group III, and the conclusion must inevitably be drawn that the greater amount of total energy in the ration of group III has resulted in a better fattening of the animals without, however, improving the real growth of the pigs.

Comparing groups I and II quite a different aspect is obtained as apart from the one period in which pig 12 during the first experiment was used, the greater gain in weight per day of the animals of group I is accompanied by a greater gain in protein. This shows that the larger amount of energy given to the first group was used in the first instance to save protein.

EXPERIMENT II.

A second experiment with only one pig in every group, the pigs being all barrows and litter-mates, was conducted to investigate how far the same amount of protein as in the periods III–VI of the former experiment with increasing amounts of energy would give better results in daily increase of weight. As these pigs were about 4 weeks' older than the other pigs, they received during the first two periods the same ration as the other pigs during periods III–VI, and received afterwards during three periods rations in which the protein content was not altered very much (apart from changes in the digestibility of the rations which could not be determined before the experiment was started) but in which the total energy was much larger. The first two periods are thus directly comparable with the four last periods of Exp. I.

Table V.

Pig No.	Periods	Total amount of protein consumed in gm.	Total amount of protein in faeces in gm. (N × 6·25)	Total amount of protein digested in gm.	% digestion
12	III, IV, V, VI	206·6	35·6	171·0	82·8
23	I	206·6	63·3	143·3	69·4
23	II	206·6	55·6	151·0	73·1
16	III, IV, V, VI	197·8	34·3	163·5	82·7
9	III, IV, V, VI	197·8	34·8	163·0	82·4
21	I	197·8	23·6	174·2	88·1
21	II	197·8	29·8	168·0	84·9
10	III, IV, V, VI	325·9	93·1	232·8	71·4
19	III, IV, V, VI	325·9	65·8	260·1	79·8
22	I	325·9	68·3	257·6	79·0
22	II	325·9	70·0	255·9	78·5
18	III, IV, V, VI	290·7	50·7	240·0	82·6
13	III, IV, V, VI	290·7	55·1	235·6	81·0
20	I	290·7	53·4	237·3	81·6
20	II	290·7	52·6	238·1	81·9

Table V gives the average digestibility of the pigs of the first experiment and of the four pigs used in this experiment, and it is evident that the digestibility of the same ration differed little between the pigs of this and the other experiment.

In Table VI the most important results obtained are shown.

Table VI.

Pig No.	Period	Digested protein in gm.	Retained protein in gm.	% retention	Total S.E. in gm.	Ratio protein : S.E.	Increase in weight per day in gm.
23	I–II	146·7	98·9	67·4	982	1 : 6·7	388·8
21	I–II	171·1	83·7	48·9	687	1 : 4·0	291·6
22	I–II	256·8	160·8	62·6	1466	1 : 5·7	453·6
20	I–II	237·7	129·2	54·4	1010	1 : 4·2	405·0
23	II, IV, V	133·7	81·3	60·8	1220	1 : 9·1	507·6
21	III, IV, V	174·3	120·4	69·1	888	1 : 5·1	324·0
22	III, IV, V	262·4	162·7	62·0	1832	1 : 7·0	648·0
20	III, IV, V	259·0	144·1	—	1402	1 : 5·4	442·7

Comparing the figures of pig 23 with those of pig 21 during periods I and II, the poor gain in weight of the last pig as well as the poor percentage of retention of protein confirm what was found with pigs 16 and 9 in the periods III and IV, viz. that the amount of energy was totally inadequate to promote normal growth, and that protein was broken down to provide fuel for the body. Although pig 23 showed a high percentage retention of protein its gain in weight is, as was the case with pig 12 in the previous experiment, insufficient; which shows again that about 1 S.E. was not enough to provide for the normal development of a young animal at this stage.

Increasing the amount of protein, as was done for pig 20 in comparison with pig 23, but keeping the energy at the same level, has not resulted in a greater gain of the body, but brought the percentage retention of protein down as it did to pig 18 in the previous experiment.

As soon as the energy was increased to 1·417 S.E. and the digested protein amounted to about 255 gm., a normal increase in weight was established as is shown by pig 22.

The periods I and II of this experiment thus confirm all that has been found in the preceding experiment during the periods III–VI. Without receiving rations which contained more protein, the pigs were kept another three periods in which, however, the energy of each ration was considerably higher than that of the previous ration. Pig 23, digesting even a little less protein during these periods than during periods I and II, received 1·22 S.E., and its increase in weight, amounting to 508 gm. per day during 3 weeks, is quite satisfactory and is even more than that of pig 20, which received 1·4 S.E. and about 85 per cent. protein more.

Pig 22 receiving the same amount of protein as in periods I and II but 1·83 S.E. makes large gains and proves that a total amount of energy of 1·8 S.E. besides 260 gm. digestible protein is ample to support normal growth.

If the results obtained in the first experiment with group III and in the second experiment with pig 22 may be considered to represent the lowest limit of increase in total live weight and increase and percentage retention of protein, we may conclude that for pigs, weighing about 100 lb. or 45 kg., an amount of 200–220 gm. protein and 1·2–1·3 S.E. is sufficient, while for pigs of about 130 lb. or about 60 kg. 230–250 gm. protein and 1·7 S.E. would be about the standard for feeding rationally.

The percentage retention of protein in such rations as mentioned above could be estimated as varying between 60 and 65, although some individuals show figures during certain periods as low as 56 and as high as 71.

EXPERIMENT III.

Guided by the figures of the preceding experiments it was decided to conduct a third experiment in which pigs of about 35–40 kg. were divided into two groups, the one group receiving during 3 weeks a ration containing a little more S.E. than was considered normal with regard to the previous experiments and with about the standard amount of protein, the other group receiving about the same S.E. in the ration and about 35 per cent. more protein.

The animals were kept for 3 weeks on this ration. At the beginning of the fourth week the ration was changed by only increasing the protein in the first group so much that it corresponded with the standard prescribed for the weight the animals had reached after the first 3 weeks, while the total S.E. were a little lower than those which had been found to be sufficient in the previous experiments. The other group was again fed with about 25 per cent. more protein and received slightly more S.E. than was considered necessary. This ration was given during 4 weeks, the week of change of ration included.

The animals were all pure-bred Tamworth pigs, were litter-mates and were barrows. Two animals were placed in each group, the weights at the beginning of the feeding of the first ration being between 35 and 40 kg., and at the beginning of the period of the feeding of the second ration being between 50 and 57 kg.

As the determination of the digestibility and the retention of the protein in trials of 48 hours' duration was apt to introduce small errors, which, however, do not always neutralise one another even when the results of more periods of 48 hours are averaged, it was decided to leave the pigs on the first ration in the boxes during the first and the last 7 days of the trial, which lasted 21 days. After the week in which the rations were changed, the pigs went in again for 7 days, remained 7 days free in the stable and went in again the last 7 days of the trial with the second ration.

Each period of 21 days consisted thus of a stay of 14 days in the boxes and a stay of 7 days in the ordinary pens.

The figures collected in connection with the digestibility and retention of protein are for this reason much more accurate, but unfortunately it was shown during this experiment that the stay in the boxes often interfered in a peculiar way with the increases of live weight, which will be discussed later on. To make the increases comparable the total increase in weight during the 3 weeks was calculated, and this increase divided by three was compared with the average consumption, digestion and retention of food during the 3 weeks as determined during the weeks the animals remained in the boxes.

The mixture of food given consisted of pollard and maize meal, whereto skim milk and blood meal were added to enable the animals to consume the fairly high amounts of protein. The rations were, however, calculated in such a way that every animal got all four kinds of food to prevent differences in retention through changes in the biological value of the different proteins in the different kinds of feed.

As the total amounts of faeces and urine grew rather too large to be kept for sampling, the faeces were weighed every day, after which 10 per cent. was directly preserved with formalin, and the urine was measured every day and also 10 per cent. was kept in large, airtight, glass-stoppered bottles after having been preserved with toluol.

After 7 days, the faeces were well mixed and average samples were taken, the same being done with the urine. All analyses were made in triplicate and the average taken as the right figure provided the differences between the analyses was within the usual limits.

The figures for the digestibility of the crude protein as given in Table VII show that the different determinations of the digestibility as done by collecting the faeces during 7 days vary only very slightly, which permits one to take the average of these determinations for every pig and every ration when comparing all the periods together.

It is interesting to note that all the pigs digested less protein during the first 7 days in the boxes than during the second periods of 7 days, which may be attributed to their not being used to the procedure of the digestion trial.

Table VII.

Pig No.	Period	Total protein consumed per day in gm.	Total protein in faeces gm.	Total protein digested per day in gm.	% digestibility
1	II	237·2	32·5	204·7	86·3
2	I	237·2	36·7	200·5	84·5
2	II	237·2	27·3	209·9	88·5
3	I	322·9	47·4	275·5	85·3
3	II	322·9	32·6	290·3	89·9
4	I	322·9	58·3	264·6	81·9
4	II	322·9	32·7	290·2	89·9
1	III	282·1	26·6	255·5	90·6
1	IV	282·1	30·1	252·0	89·3
2	III	282·1	28·7	253·5	89·9
2	IV	282·1	34·1	248·0	87·9
3	III	375·3	44·5	312·8	87·5
3	IV	357·3	37·7	319·6	89·4
4	III	357·3	35·7	321·6	90·0
4	IV	357·3	39·3	316·0	88·4

Table VIII.

Pig No.	Weight beginning of week, lb.	Weight beginning of following week, lb.	Gain or loss in lb.	Remarks
1	94·25	107·75	13·50	In box
1	107·75	116·00	8·25	Out of box
1	116·00	119·00	3·00	In box
1	119·00	133·00	14·00	Out of box
1	133·00	137·00	4·00	In box
2	92·00	104·75	12·75	In box
2	104·75	105·00	00·25	Out of box
2	105·00	119·75	14·75	In box
2	119·75	129·00	9·25	Out of box
2	129·00	133·00	4·00	In box
2	133·00	146·00	13·00	Out of box
2	146·00	154·00	8·00	In box
3	89·25	104·25	15·00	In box
3	104·25	106·75	2·50	Out of box
3	106·75	119·75	13·00	In box
3	119·75	127·00	8·25	Out of box
3	127·00	131·00	4·00	In box
3	131·00	149·00	18·00	Out of box
3	149·00	147·00	2·00	In box
4	77·75	92·00	14·25	In box
4	92·00	92·00	—	Out of box
4	92·00	105·50	13·50	In box
4	105·50	110·00	4·50	Out of box
4	110·00	119·00	9·00	In box
4	119·00	126·00	7·00	Out of box
4	126·00	130·00	4·00	In box

The individual differences in digestibility amongst the pigs are negligible. The animals all behaved well and no disturbances in health occurred during the experiment.

In Table VIII the weights of the different animals at the beginning of each week are recorded together with the gains which the animals made during the weeks.

As can be seen from this table, all the pigs show the same peculiarities in gain during the trial. During the 3 weeks in which they received the first ration, all the pigs made exceedingly large gains when in the boxes and during the week they were out made practically no gain at all. The weather was fairly cold, and as the pigs remained in a large well-ventilated byre on the cold concrete floor when out of the digestion boxes, it can be easily understood that a large amount of energy was used to provide for their maintenance. During the 3 weeks in which the pigs received the second ration after having been changed over to the new ration during the week, which is shown in the tables between lines, the reverse occurred. The gains made during the stay in the boxes were very small compared with those made when outside the boxes, which was perhaps due to the hot oppressive weather prevailing during that time probably causing the pigs in the narrow boxes to suffer more than when outside in their pens.

This may illustrate the dangers run in relying on figures about the gain of weight made in experimental apparatus, and the only way in which these dangers can be to a certain extent excluded is to calculate the average increase in weight per day during the first 3 weeks and during the last 3 weeks and to compare these figures with the average digested protein and the average retention shown during the weeks when the animals stayed in the boxes.

This has been done in Table IX, which gives the average retention of protein per day, the percentage retention, the digested protein, and the average increase in weight per day, as also the total energy of the ration and the ratio between energy and digested protein.

Table IX.

Pig No.	Periods	Digested protein per day in gm.	Retained protein per day in gm.	% retention	Total S.E. per day	Ratio digested protein : S.E.	Increase in weight per day in gm.
1	II	204·7	136·0	66·4	1·249	1 : 6·1	875*
2	I, II	205·2	136·9	65·0	1·249	1 : 6·1	599
3	I, II	282·9	157·0	55·3	1·239	1 : 4·4	659
4	I, II	277·4	185·1	66·7	1·239	1 : 4·5	610
1	III, IV	253·7	216·3	85·3	1·492	1 : 5·9	454
2	II, IV	250·7	174·0	69·4	1·492	1 : 5·9	497
3	III, IV	316·2	205·8	65·1	1·565	1 : 4·9	432
4	III, IV	318·8	221·1	69·4	1·565	1 : 4·9	432

Only one period in which the increase in weight was taken.

The figures reveal in the first instance that the increase in protein from the first to the second ration did not interfere with the percentage retention except in the case of pig 1, which showed an abnormally high retention during the 3 weeks in which it received the second ration. Pig 3 has a somewhat low retention during the periods of the first ration, which is, however, not at all abnormal.

The gains in weight during the periods of the first rations show that the total amount of energy was quite sufficient.

The increase in protein in the second ration, judged by the consistency of the percentage retention, may be considered sufficient, but the fall in increase in weight shows that the total energy of the food is just about the lowest limit one could accept for pigs of this age. The group of pigs which received about 35 per cent. more protein with the same amount of total energy did not show a smaller percentage of retention and cannot be regarded in this respect as having a superfluous consumption of protein. The gains in weight are, however, about the same, and even in some periods lower than those of the animals in the group with the lower amount of protein, from which it may be concluded that more nitrogenous body substance per unit of weight was retained in the second than in the first group.

It must, however, be borne in mind that although the percentage retention did not change and the higher gain of protein may have a preference with regard to the quality of the animals as baconers, this higher gain involved at the same time a higher waste of protein as shown by the higher excretion of nitrogen per day in the urine.

It becomes therefore a matter of economic importance whether the enrichment of the body in protein without an adequate gain in live weight as occurs by feeding excessive amounts of protein in a ration is a profitable procedure and can be recommended to producers of baconers.

As far as standards of feeding can be deduced from this experiment, the first ration with the lower amount of protein may be considered about adequate on account of the gains in live weight.

A standard of 200–220 gm. digested crude protein, necessitating 250–275 gm. total crude protein in the ration (if 80 per cent. of the protein is digestible) with a total energy of 1·2–1·3 starch units, seems to be about the desirable amount for pigs of about 40 kg. The animals, to make the same gain during the time they received the second rations, might perhaps have received more total energy in this ration and the amount of 300–325 gm. total protein, corresponding with 250–270 gm. digested protein and 1·6–1·8 starch units, represents more or less the lowest amount of food the animals should receive when weighing up to 60 kg.

CONCLUSIONS.

From the figures of retention of protein and from the increases in live weight the animals made when different amounts of starch units were fed, it may be concluded that increases in weight alone do certainly create an inaccurate basis on which to formulate feeding standards for growing pigs.

The increases in weight have to be controlled for their normal proportion of increase in protein and in fat either by determining the nitrogen balance of the animal or by judging the normal composition of the animal when slaughtered.

The thickness of the layer of fat on the back and the estimation of the normality of the meat requiring a good marbling of these parts has been the criterion for a normal growth and condition in the Danish and Swedish experiments, while total analyses of fattened animals compared with those of control animals have formed the basis of different American experiments to ascertain whether the growth of the animal had been normal.

As the first method could have been followed but would have taught us nothing about the retention of protein, while the second method was impossible through lack of equipment, the method explained in this paper was introduced, namely a determination of the increase in nitrogenous matter in the body combined with the determination of the total increase.

The best method to be followed will always be the determination of a complete carbon, nitrogen and mineral balance of the animal, but in the absence of respiration apparatus the application of this method was impossible.

The comparison of the nitrogen balance with the increases in weight has shown that a lack of non-nitrogenous substances in the ration reflects itself not only in a low total gain in live weight, but often also in a very low retention of the protein of the ration, part of it being broken up to provide energy for the growing body. A certain proportion of protein to total energy in a ration for growing animals seems paramount for the normal development of such animals.

Fairly high increases in live weight may be obtained in growing animals by feeding superfluous amounts of non-nitrogenous matter, but it appears that these increases do often lack the corresponding amount of protein, as the percentage retention of protein cannot be increased indefinitely.

When a retention of 60–70 per cent. of the daily digested protein is reached, it is very improbable that an increase in the amount of protein or the amount of energy can still improve this percentage retention. When the retention remains, however, on the same level, a stage is reached in which the heavier losses of nitrogenous matter through the urine make the profitableness of the ration very doubtful.

When more protein is given than can be physiologically absorbed by the growing body at a rate of 60–70 per cent. retention, this percentage retention will fall and a superfluous consumption of protein will set in.

Whether the percentage retention of energy is not changed when greater amounts are given than are necessary for an increase in weight up to about 700 gm. per day will have to be determined in the future.

But even when the percentage retention of total energy did not suffer materially to a very great extent, it must also be borne in mind that increases in body weight nearing the physiological limit are only made at the cost of a greater loss of energy at the same time, a loss which may have a detrimental influence on the economic aspect of the suitability of the ration.

As far as these researches can help to determine the maximum amount of protein a normal growing animal is able to retain daily, it is found that, omitting the extremes, this amount lies in these experiments between ± 120 and 150 gm. (according to individual differences) for pigs of 40–50 kg., between ± 140 and 170 gm. for pigs of 50–60 kg. and can increase to ± 160–200 gm. per day for pigs weighing 60–70 kg., provided the daily increase in live weight of these pigs varies between 550 and 650 gm. during a reasonably long period (3–4 weeks).

Taking, however, the following increases in protein and live weight per day as being quite satisfactory and considering a 60–65 per cent. retention as a fair average, the standards for the amount of protein can be easily calculated as follows:

Weight of pig in kg.	Increase in protein in gm.	Increase in weight in gm.	Required digestion protein in gm.	
			60 % retention	65 % retention
40–50	120	550	200	185
40–50	140	650	230	215
50–60	140	550	230	215
50–60	160	650	270	245
60–70	160	550	270	245
60–70	180	650	300	280

In the column of the required protein the necessary amount of digestible protein has been calculated with a 60 per cent. retention as standard and with a 65 per cent. retention as standard.

As the researches of Hansson and of C. S. Schmidt show that besides individual variations different breeds vary in their retention of protein during growth, it must be pointed out that these amounts are determined in experiments with Large Black × Tamworth crosses and with pure-bred Tamworth, being breeds with a quick growth especially suited for the production of baconers.

The amount of energy necessary to obtain this growth can only be estimated from the calculations of the S.E. of the different rations and from the results obtained with these rations, although it is impossible to decide whether the percentage retention of total energy in the body had reached a normal level.

Unfortunately the increases in live weight during the first experiment were generally too low to permit conclusions in this direction. From the figures of the second experiment, it may be deduced that 1·20 S.E. are sufficient for a normal increase in weight of a pig of about 40 kg. For pigs between 40 and 50 kg. more figures are available, which show that at least 1·5 S.E. had to be provided for good growth in our experiments. For pigs of 50–60 kg., those receiving 1·45 S.E. did not show sufficient gain at all, and this was hardly reached when between 1·5 and 1·6 S.E. were given. Even the weights attained in giving these amounts indicate that a higher amount of total energy, up to 1·8 S.E., could have been given profitably.

For pigs heavier than 60 kg. the figures of the second and third experiments may give some indications as to the effect that rations containing up to 2·1 S.E. for pigs between 60 and 70 kg. may have in promoting a good growth, though the higher limit should certainly be considered to be 2·2 S.E. per day.

Comparing the figures thus obtained from these retention trials and weighings with the figures of other authors obtained in numerous practical experiments, the figures of Hansson and of Clausen are the most accurate, and are compared with our figures in the table below. As our pigs were usually about 40 kg. when entering the trials, attention must be drawn to the standards for pigs between 30 and 40 kg., which are more or less arbitrary, being arrived at by deduction from the figures of heavier animals, while the figures for pigs between 60 and 70 kg. are based only on a few observations, as most of the animals in these experiments weighed between 40 and 60 kg.

Pig weights in kg.	Digested protein per day in gm.	S.E. per day	Feed units per day*	Gm. protein per S.E.	Authors
30–40	180–220	1·05–1·33	1·5–1·9	165–171	Hansson, C.
30–40	215	1·26	1·8	170	Clausen
30–40	200–225	—	—	—	„
30–40	190	1·2	1·7	158	Reimers and Bartel
30–40	180–200	1·1–1·3	—	—	„ „
40–50	220–250	1·33–1·61	1·9–2·3	155–165	Hansson, C.
40–50	245	1·61	2·3	152	Clausen
40–50	235–255	—	—	—	„
40–50	215	1·5	2·14	144	Reimers and Bartel
40–50	200–230	—	—	—	„ „
50–60	250–270	1·61–1·82	2·3–2·6	148–155	Hansson, C.
50–60	265	1·855	2·65	143	Clausen
50–60	255–275	—	—	—	„
50–60	250	1·8	2·43	139	Reimers and Bartel
50–60	230–270	1·7–1·9	—	—	„ „
60–70	270–280	1·82–1·96	2·6–2·8	143–148	Hansson, C.
60–70	290	2·065	2·95	140	Clausen
60–70	275–300	—	—	—	„
60–70	285	2·1	3	135	Reimers and Bartel
60–70	270–300	2·0–2·2	—	—	„ „

* The feed unit in this table is taken as equivalent to 0·7 S.E.

The figures arrived at are as a rule a little lower than those of the Danish and Swedish investigators with regard to the total amount of protein necessary for animals of these weights, while the S.E. are approximately the same as determined by these authors. It is impossible to explain this difference otherwise than by supposing that our rations might have contained even more protein per day, and might have given better results if this had been done or by supposing that the increased heat production resulting from rations with proportionally more protein may be more easily eliminated without harmful effect on the animal in some climates as the Danish and Swedish, which are colder than our climate where a high amount of protein is more inclined to upset the health of the animal.

In unpublished researches in which very high amounts of protein were given to young animals here, these amounts created such heavy sweating during hot weather that the animals certainly would have done better if the daily ration of protein had been slightly reduced.

It is also possible that the animals used are of a rather late maturing type and are not capable of making such enormous gains when young as quicker maturing animals can do, which would suggest that instead of the amount of the protein being too small, the amounts of S.E. were rather too large and could have been slightly decreased.

Further researches on these and similar questions arising during these trials are at present in progress at this Institution.

SUMMARY.

1. Exp. I was conducted with two pigs in each group, which pigs were kept during certain times in digestion boxes, permitting the separate collection of faeces and urine.

2. The total amount of food given to the pigs was slightly below the present standards.

3. The different rations contained different amounts of protein and different amounts of non-nitrogenous constituents.

4. Exp. II was conducted with one pig in each group and served to control and supplement the data collected in the previous experiment.

5. Exp. III was done with two pigs in each group. Various alterations in the technique were introduced and the total amounts of food per day exceeded slightly the present standards.

6. The retention of protein during the different experimental periods is given in the tables and the percentages of retention are calculated.

7. From these data the authors calculate the standards for protein at different ages of the animals.

8. With the aid of the figures of the increase in weight per day of the growing animals, the standards for non-nitrogenous constituents are estimated and expressed in starch equivalents.

9. The standards thus obtained are compared with those of other investigators.

10. Further experiments have been planned to collect a larger number of data from which standards may be calculated.

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(*Received March 5th,* 1935.)

THE IMPROVEMENT OF WINTER OATS.

By H. HUNTER, M.A., D.Sc.

(Plant Breeding Institute, School of Agriculture, Cambridge.)

A CONDITION militating against the successful cultivation of spring oats in south-eastern districts in England is the limited rainfall in the early months of the year. The initial effect of a deficiency of moisture is to retard the vegetative development of the plant, and this alone may be sufficient to affect the subsequent yield of grain and straw adversely. A more serious concomitant effect is that it renders the crop liable to the ravages of frit-fly (*Oscinella frit*). The damage caused by this pest is frequently measurable in a loss of half the crop, whilst in the event of more serious attacks the whole crop may fail.

The extent of damage resulting from an attack may be modified by early sowing, for this ensures the development of the plant beyond its most vulnerable period which coincides with the second, third and fourth leaf stage, before the first generation of the fly appears about the end of April (3, 4).

Favourable weather for drilling, however, is not within the control of the cultivator, and early sowing is thus only a possible security. A more reliable and effective control is that offered by autumn sowing, for in this case the plants have advanced considerably beyond the stage of acutest vulnerability before the fly becomes active in the spring.

Probably all the varieties usually regarded as spring sorts can survive the normally temperate winters experienced in this country, but they are totally unsuited for the more rare seasons with winter conditions of a more extreme character. In practice, the more satisfactory and safer procedure is the use of those varieties which have been demonstrated to possess a high degree of resistance to severe winter conditions. The number of such varieties available, however, is severely limited, for only two—Grey Winter and Black Winter—come into this category. Both of these varieties are characterised by prostrate habit of early growth, high tillering capacity, dark green, long, narrow leaves, and by a quality of grain which, with few exceptions, is superior to that of the generality of spring varieties. The feature common to the two oats operating against their more extended use in this country is the straw, which is long, fine, and extremely liable to "lodge", particularly immediately preceding and following flowering.

The account which follows presents some of the results of one of a series of breeding experiments designed as an attempt to secure hybrid varieties possessing a sufficiently high degree of winter hardiness for the British Isles, associated with good standing straw. As Grey Winter is slightly more winter hardy than Black Winter, and is also characterised by grain of somewhat higher quality, it was used as the winter-hardy parent in these investigations, and since there is no winter variety with good straw available, the other parent was a spring variety denominated "Argentine" (*Avena sativa*) because it was selected from the produce of a parcel of seed originating from that country. In early growth habit Argentine is semi-erect to erect; its tillering capacity is inferior to that of Grey Winter, but superior to that of Abundance and Victory; its leaves are longer, narrower and more pendulous than in the two latter varieties. The Argentine straw is from 30 to 40 cm. shorter than that of Grey Winter, but distinctly superior in standing power at all stages of development, and when fully ripe it does not become brittle very readily. In addition to the more obvious differences in length, the straws of the two oats are also readily distinguishable in their respective degrees of "fineness"—a term here employed to describe the diameter of the culm taken at any point throughout its length relative to similar measurements made at corresponding positions in other varieties; on this basis Argentine straw having a substantially greater diameter is described as "coarse", and Grey Winter as "fine".

Since the shape of the oat grain may be utilised as one of the few physical indexes of its quality, this feature in the case of the two parent varieties should be noted. Grey Winter grain is long (10–11 mm.) and inclined to be spindle-shaped, that is, its greatest width is slightly less than half-way between the base and apex of the grain; Argentine, on the other hand, is somewhat shorter than Grey Winter, with sides more nearly parallel throughout their whole length. The ventral palea in Grey Winter is convex, whilst in Argentine it is generally more flattened, and at times slightly concave. The convex or concave curvature of the inner palea is an intimate reflection of the shape of the ventral surface of the caryopsis; thus, in Grey Winter the ventral groove is almost completely filled, and in cross-section the caryopsis is nearly circular, whilst in Argentine there is frequently a distinct hollow in the upper portion of the ventral surface. These differences, although small, appear to be closely associated with low husk content in varieties simulating Grey Winter, and with a higher husk content in varieties with features corresponding to those exhibited by Argentine.

Qualitatively, Grey Winter and Argentine grain exhibit important differences which are apparent in a series of chemical determinations made in 1928. The figures refer to the composition of the kernel and the whole grain, and are the average of a series of three comparative plots of each of the two varieties grown in 1927–8.

	Protein %	Ether extract %	Carbo-hydrates %	Crude fibre %	Ash %	Husk % of whole grain	1000-grain weight gm.
			(1) Kernel.				
Grey Winter	11·12	10·45	76·36	—	2·07	—	—
Argentine	12·09	8·68	77·09	—	2·14	—	—
			(2) Whole grain.				
Grey Winter	9·58	8·34	70·72	7·94	3·43	20·7	36·8
Argentine	9·63	7·42	67·49	12·65	2·82	28·9	37·6

The kernel figures show a small superiority of Grey Winter in ether extract, but concurrently there is a small difference in protein in favour of Argentine. Other determinations made in the course of the investigations have shown a similarly high value in ether extract in Grey Winter and have, in addition, provided further evidence that this constituent varies inversely with the amount of protein. Consequently, although the differences in composition observed in the case of Grey Winter and Argentine may be regarded as demonstrating true varietal features, they are insufficient to differentiate the two varieties qualitatively.

In the whole grain, on the other hand, the crude fibre provides a measure of differentiation absent in the hull-less samples.

A portion, amounting approximately to 1 per cent., of the total amount of crude fibre of the whole grain, is derived from the caryopsis, but the main part is contributed by the husk, of which fibre constitutes a third of the whole weight. Qualitatively, oat husk partakes of the character of a coarse fodder, to which it bears a further likeness in so far as the fibre it contains is not pure cellulose, but cellulose encrusted by silica and ligno-cellulose. As energy beyond anything it may contribute itself is required to effect the digestion of such material, its value as a food is confined to the benefit it may confer on the grain by facilitating the process of mastication.

The results of numerous feeding experiments furnish evidence of the influence of crude fibre in determining the digestibility of nutrient substances, but only two will be quoted here as illustrative of a general effect. The first is one carried out by Woodman, Evans and Menzies Kitchin (11) in feeding swine, and in this case Sussex-ground oats, ordinary farm-ground and crushed oats were compared; the first contained 13·3 per cent., the second 12·9 per cent. and the third 11·18 per cent. of crude fibre. The digestion coefficients obtained were:

	Dry matter %	Organic matter %	Crude protein %	Ether extract %	N-free extract %	Crude fibre %
Sussex-ground	73·6	75·9	75·0	84·1	81·2	47·1
Farm-ground	66·1	67·5	85·5	86·8	74·3	0·0
Crushed	54·7	56·7	68·6	63·8	65·9	0·0

and the amounts of nutrients digested per 100 lb. of dry matter consumed were:

	Total organic matter lb.	Crude protein lb.	Ether extract lb.	N-free extract lb.	Crude fibre lb.
Sussex-ground	72·72	9·99	5·44	57·13	6·16
Farm-ground	68·42	11·03	4·67	49·72	0·00
Crushed	55·22	7·56	3·50	44·05	0·00

Here the fineness of division obtained in Sussex-ground oats has greatly improved the value of the oats when fed to this particular class of stock; the results also emphasise the predominant effect of the crude fibre in determining the relative feeding values of the three descriptions of oats.

The second series of results are those obtained by feeding three varieties of oats to poultry. The varieties were Grey Winter with 23 per cent. of husk, Bountiful with 25 per cent., and Potato with 29 per cent. Halnan (6) found that in Grey Winter, with a fibre content of 8·7 per cent., the digestive coefficient of the organic matter was 68·6 per cent., and that of the N-free extract 76·5 per cent. The corresponding figures for Bountiful, with 9·5 per cent. of fibre, and of Potato with 11·4 per cent., were for organic matter 62·8 and 55·8 per cent. and of N-free extract 68·5 and 66·7 per cent. respectively. The results demonstrate, as Halnan observes, that the digestibility of the organic matter and N-free extract is clearly affected by the fibre content of the oat, the presence of fibre in the oat having a depressant effect on the digestibility of these two nutrients. This depressant effect is not shown, however, in the case of the protein and the fat. Translated into practice these results mean that "from the point of view of the poultry farmer...a ton of Grey Winter oats contains 108 lb. more digestible matter than a ton of Bountiful oats and no less than 238 lb. more digestible matter than a ton of Scotch Potato oats".

As Woodman (10) points out, however, in evaluating these and other results of feeding experiments due allowance must be made for the extent to which fibre exists in a food in a non-lignified or a lignified condition, and then for the character of the consuming animal. In oats the fibre exists in a lignified condition, and as such its digestibility is not high with any class of farm stock; it appears, however, to reach a maximum with sheep and remains at a minimum with swine and poultry.

Whilst the differences in husk content between Grey Winter and Argentine in the examples given above are large enough to rule out the probability of their being due to fluctuation, this is a possibility which must not be overlooked. In order to determine some of the conditions which may operate to give rise to differences in this feature from year to year, a series of plots was sown yearly for a period of five years, 1927–32. Twelve varieties were included in the series and the seed used in the case of every variety was a portion of the produce of a single plant culture of the particular variety. The seeds were sown singly at distances of 2 in. apart in the drills, which were 6 in. apart. Thus, the initial plant population per unit length of drill was identical for every variety throughout the whole period of the investigations.

The plots were sown in the autumn of each year, and an exact replica in the succeeding spring. In addition to providing information on the question under immediate review, at least two other comparisons were thereby made possible, namely, first the effect of autumn and spring sowing on the husk content, and secondly the degree of resistance of each of the twelve varieties to winter conditions. The latter is a feature which is dealt with in greater detail later but it may be noted here that the cereal year 1928–9 is excluded, since all but the true winter varieties were destroyed by the severe cold experienced in January and February, 1929.

Table I. *Showing the percentage of husk in twelve varieties of oats*, 1927–32.

(*a*) Autumn sowings.

Harvest year ...	1927	1928	1930	1931	1932	Average for 5 years
Grey Winter	20·2	20·6	20·7	20·1	22·9	21·0
Avoine d'hiver	21·6	21·8	21·2	21·2	24·6	22·1
Gris de Houdan	21·6	22·0	21·5	21·1	22·1	21·6
Sandy	22·4	22·0	21·7	22·6	24·3	22·6
Hopetoun	24·5	23·4	22·1	23·3	22·9	23·2
Ligowo	24·0	24·6	25·7	24·4	24·3	24·6
Abundance	23·7	24·6	26·1	24·4	27·0	25·2
Bountiful	26·1	26·4	25·4	26·4	26·8	26·2
Potato	26·7	27·0	26·1	26·4	24·8	26·2
Argentine	25·1	29·0	28·2	27·5	30·8	28·1
Black Tartary	30·2	29·3	29·5	28·6	29·3	29·4
Yielder	32·8	32·3	31·4	33·0	33·8	32·7
Average	24·9	25·3	25·0	24·9	26·1	

Table II.

(*b*) Spring sowings.

Harvest year ...	1927	1928	1930	1931	1932	Average for 5 years
Grey Winter	22·3	20·8	21·0	21·3	20·4	21·2
Avoine d'hiver	21·6	21·3	20·7	22·7	21·5	21·6
Gris de Houdan	22·4	20·9	21·2	21·3	20·9	21·3
Sandy	23·3	21·2	23·4	23·3	23·1	22·8
Hopetoun	23·8	23·4	22·6	24·7	21·0	23·1
Ligowo	24·9	24·5	25·5	23·7	25·4	24·8
Abundance	25·1	25·8	25·0	24·1	26·8	25·4
Bountiful	25·9	25·6	26·2	26·3	27·4	26·3
Potato	26·7	27·0	26·0	27·4	23·1	26·0
Argentine	27·6	29·4	27·3	28·2	29·7	28·4
Black Tartary	31·3	29·4	29·5	28·8	28·7	29·5
Yielder	37·0	33·3	32·5	31·8	33·4	33·6
Average	25·9	25·2	25·0	25·3	25·1	—

Table III. *Showing the* 1000-*grain weight* (*gm.*) *in twelve varieties of oats*, 1927–32.

(*a*) Autumn sowings.

Harvest year ...	1927	1928	1930	1931	1932	Average for 5 years
Grey Winter	40·9	37·5	37·2	38·2	30·8	36·9
Avoine d'hiver	40·8	40·1	40·5	42·3	35·4	39·8
Gris de Houdan	33·1	30·3	33·0	33·4	38·9	31·7
Sandy	28·4	27·1	27·0	30·5	28·1	28·2
Hopetoun	30·1	29·1	27·7	30·3	25·1	28·5
Ligowo	42·4	38·6	36·2	40·7	37·4	39·0
Abundance	40·0	38·6	33·1	44·9	33·3	38·0
Bountiful	45·5	39·7	48·2	48·8	41·1	44·6
Potato	31·2	29·6	29·5	31·1	28·5	30·0
Argentine	40·5	35·6	35·7	44·3	31·8	37·6
Black Tartary	34·2	32·4	32·0	34·8	29·3	32·5
Yielder	40·0	37·7	32·9	40·8	37·9	37·9
Average	38·1	34·7	34·4		34·8	

Table IV.

(*b*) Spring sowings.

Harvest year ...	1927	1928	1930	1931	1932	Average for 5 years
Grey Winter	40·0	37·5	38·2	38·8	37·8	38·5
Avoine d'hiver	41·0	41·7	43·8	43·4	42·4	42·5
Gris de Houdan	34·7	32·8	33·9	34·4	30·3	33·2
Sandy	29·9	29·4	28·0	32·5	31·5	30·3
Hopetoun	30·8	29·0	29·5	31·0	26·7	29·4
Ligowo	39·8	36·9	36·9	43·7	37·1	38·9
Abundance	39·3	37·2	36·9	42·8	35·4	38·3
Bountiful	44·9	41·8	46·6	47·0	41·5	44·3
Potato	29·8	30·6	29·6	31·1	28·8	30·0
Argentine	36·2	36·2	39·0	43·4	33·5	37·7
Black Tartary	32·1	32·8	32·6	35·8	29·6	32·6
Yielder	43·9	39·5	39·4	43·4	39·5	41·1
Average	36·8	35·5	36·2	39·6	34·5	—

The comparisons are amplified by the inclusion throughout of 1000-corn weights and these are presented in Tables III and IV.

In the harvest year 1932, several of the varieties, notably Grey Winter, Avoine d'hiver, Sandy, with Abundance and Argentine which suffered from contiguity with these three lots, were badly "lodged" early in the season, and the husk figures in these cases are above the respective averages of these varieties for the 5-year period. With these exceptions the series of varieties exhibits a remarkable uniformity throughout the experimental period, a result, it will be noted, that applies to varieties exemplifying a gradation of values between 21 per cent. at one extreme to approximately 33 per cent. at the other.

The 1000-grain weights on the other hand exhibit wide seasonal fluctuations, more in some varieties than in others; the increase or decrease of grain weights, however, does not appear to exert a corresponding effect on the percentage of husk. The two varieties Grey Winter and Argentine provide a good example of this, for whilst there is a significant difference of 7 per cent. in their husk content, there is no difference in their respective 1000-grain weights.

It will also be observed that in the case of every variety the percentage of husk is unaffected by the season of sowing.

It may be claimed that the rate of seeding adopted in this series of comparisons has influenced the results, and that with a larger number of plants per unit length of drill the percentages of husk would tend to be lower. Inversely, it may be sustained that if the number of plants per unit length of drill were still further reduced even higher percentages of husk would be obtained. To test this an additional plot of Grey Winter was included in both the autumn and spring sowings throughout the 5-year period, and in this plot the individual grains were sown 4 in. apart in the drill, *i.e.* at double the distance employed for the remaining plots. The average results obtained for the five were as follows:

Spacing of seed in.	Husk, % of whole grain	
	Autumn sown	Spring sown
2 × 6	20·8	21·0
4 × 6	20·5	20·8

Thus, in the case of this one variety the lower rate of seeding has no measurable effect on the percentage of husk.

It will be noted that throughout the comparisons the low percentage of husk in Grey Winter is an outstanding feature; indeed in so far as the twelve varieties can be regarded as representative of those in general use in this country, Grey Winter provides a standard of high quality.

Table V. *Showing the value of the individual differences between Grey Winter and eleven varieties in* (1) *husk content, and in* (2) 1000-*grain weight.*

(1)

	Mean difference %	*σ*	*t*	*P*
	(*a*) Spring sowings			
Grey Winter—Avoine d'hiver	+ 0·40	0·40	—	Insig.
,, Gris de Houdan	+ 0·18	0·076	2·37	>0·05
,, Sandy	+ 1·70	0·4336	3·91	>0·01 <0·02
,, Hopetoun	+ 1·94	0·4833	4·01	>0·01 <0·02
,, Ligowo	+ 3·64	0·5105	7·13	<0·01
,, Abundance	+ 4·20	0·7155	5·87	<0·01
,, Bountiful	+ 5·12	0·5462	9·37	<0·01
,, Potato	+ 4·88	0·6414	7·61	<0·01
,, Argentine	+ 7·28	0·7365	9·89	<0·01
,, Black Tartary	+ 8·38	0·2478	33·82	<0·01
,, Yielder	+12·44	0·7096	17·53	<0·01
	(*b*) Autumn sowings.			
Grey Winter—Avoine d'hiver	+ 1·20	0·1975	6·08	<0·01
,, Gris de Houdan	+ 0·76	0·407	1·87	>0·01 Insig.
,, Sandy	+ 1·70	0·279	6·99	<0·01
,, Hopetoun	+ 2·34	0·7436	3·15	<0·05 >0·02
,, Ligowo	+ 3·70	0·6099	6·07	<0·01
,, Abundance	+ 4·26	0·314	13·57	<0·01
,, Bountiful	+ 5·32	0·4332	12·00	<0·01
,, Potato	+ 5·30	0·8734	6·07	<0·01
,, Argentine	+ 7·22	0·6061	11·91	<0·01
,, Black Tartary	+ 8·08	0·4420	18·28	<0·01
,, Yielder	+11·76	0·440	26·73	<0·01

(2)

Table VI.

	Mean difference (gm.)	*σ*	*t*	*P*
	(*a*) Spring sowings.			
Grey Winter—Avoine d'hiver	+4·0	0·7849	5·09	<0·01
,, Gris de Houdan	−5·24	0·5913	8·86	<0·01
,, Sandy	−8·20	0·8613	9·50	<0·01
,, Hopetoun	−9·06	0·5573	16·26	<0·01
,, Ligowo	+0·42	1·134	0·37	Insig.
,, Abundance	−0·14	1·094	0·13	Insig.
,, Bountiful	+5·90	0·9985	5·91	<0·01
,, Potato	−8·48	0·5364	15·05	<0·01
,, Argentine	−0·80	1·631	0·49	Insig.
,, Black Tartary	−5·88	0·9805	5·99	<0·01
,, Yielder	+2·68	0·6629	4·04	<0·02 >0·01
	(*b*) Autumn sowings.			
Grey Winter—Avoine d'hiver	+2·90	0·824	3·52	>0·02 <0·05
,, Gris de Houdan	−5·18	1·068	4·85	<0·01
,, Sandy	−8·70	1·682	5·17	<0·01
,, Hopetoun	−8·46	0·851	9·94	<0·01
,, Ligowo	+2·14	1·252	1·71	Insig.
,, Abundance	+1·06	1·794	0·59	Insig.
,, Bountiful	+7·74	1·815	4·26	>0·01 <0·02
,, Potato	−6·94	1·238	5·61	<0·01
,, Argentine	+0·66	1·450	0·46	Insig.
,, Black Tartary	−4·38	0·890	4·92	<0·01
,, Yielder	+0·94	1·899	0·49	Insig.

A statistical measure of the difference between each variety and Grey Winter is given in Tables V and VI.

CHARACTER OF F_1 AND F_2 GENERATIONS OBTAINED FROM THE CROSS, ARGENTINE × GREY WINTER.

The F_1 plant obtained from Grey Winter × Argentine was semi-erect in early growth habit, the straw intermediate in length between the two parents; the grain light grey in colour and long, with a convex inner palea. The percentage of husk in the grain of this plant was 23·6, which, if the husk values of the two parents are taken at 21 and 28 per cent. respectively, is approximately intermediate between the two.

The F_2 generation was composed of 236 plants, of which 183 were grey-grained and 53 white-grained. In some cases the grey colour shaded almost imperceptibly into white, and a more accurate numerical ratio was obtained when the product was advanced to the F_3 generation, when on a colour basis the ratio obtained was:

	Homozygous grey plants	Heterozygous grey plants		White plants
	46	124		66
or	170 grey-grained		:	66 white-grained
or	2·57		:	1

Although it would be difficult to justify the procedure on a basis of feeding value, a preference for white-grained oats is frequently exercised in agricultural practice, and for this reason the further study of the hybrid progeny was restricted for the time being to the white-grained segregates. Accordingly single plant selections on the basis of good standing straw were made from white-grained families of the F_3 generation. In the following year, that is, in the F_4 generation, the most promising of these single plant selections were again retained for propagation, but at this stage the basis of selection was amplified by husk determinations.

In 1927, that is in the F_4 generation, a severe manifestation of chlorosis provided the opportunity of still further extending the scope of selection, and in that year all selections exhibiting this condition were discarded. Ultimately, having eliminated such of the selections as did not exhibit good standing straw and those with distinctly inferior grain, the material available for further examination was narrowed down to a few families, of which the most promising were those appearing under the numbers 105, 109, 136, 138, 152.

FEATURES OF HYBRID SELECTIONS.

(1) *Winter hardiness.*

The next concern was to obtain some measure of the winter hardiness of the selections contained in the chosen families.

Although various climatic and soil conditions may contribute to the conditions of resistance to winter conditions, the greatest influence is that of low temperature. The moisture content of the soil, the presence or otherwise of protective covers of snow, rapid changes of temperature, whether diurnal or of a more extended nature, are all contributory conditions, but only exert a modifying influence on the main condition of low temperature.

In geographical areas such as the greater part of the British Isles, the winter conditions in most seasons afford little opportunity of ascertaining the resistance of varieties in general use to conditions of low temperature. Occasionally a season of extra severity is encountered which distinguishes varieties with a high resistance from others with little or no resistance in an unequivocal manner.

Referring to the variety tests to which attention has been directed in reference to other features, the number of plants surviving at harvest was ascertained for all varieties in both autumn and spring sowings. These are given in detail in Table VII, and it will be observed that if for a moment the figures under autumn, 1929, are excluded, the number of

Table VII. *Showing the percentage of plants surviving at harvest*, 1927–31.

Harvest year ...	1927		1928		1929		1930		1931		Average		
	A.	S.	A.	S.	A.	S.	A.	S.	A.	S.	A.	A. excluding 1929	S.
Varieties													
(1) Grey Winter	85·7	83·0	85·2	74·3	36·5	77·8	88·7	61·7	85·7	84·3	76·3	86·3	76·3
(2) Avoine d'hiver	88·3	81·7	86·1	78·3	54·3	88·7	80·9	57·0	72·2	86·1	76·3	81·8	78·3
(3) Gris de Houdan	92·2	84·8	91·3	83·0	0	78·7	94·3	76·1	92·6	83·5	74·1	92·6	81·2
(4) Sandy	95·7	83·0	90·0	73·9	0	85·2	86·1	77·8	90·9	89·1	72·5	90·7	81·8
(5) Hopetoun	85·7	90·4	93·0	77·8	0	91·7	83·9	81·3	85·2	83·5	69·6	87·0	85·0
(6) Ligowo	83·5	80·9	88·3	74·3	0	89·6	80·9	73·0	80·0	74·8	66·5	83·2	78·5
(7) Abundance	85·7	75·7	90·4	68·7	0	80·9	77·4	65·7	67·4	77·8	64·2	80·2	73·7
(8) Bountiful	96·5	87·0	79·6	54·3	0	79·6	85·7	77·0	89·1	84·3	70·2	87·7	76·4
(9) Potato	96·1	83·9	93·9	81·7	0	85·7	83·0	76·5	84·8	82·6	71·6	89·5	82·1
(10) Argentine	90·0	81·7	85·7	64·8	0	84·3	87·0	65·7	72·6	85·2	67·0	83·8	76·3
(11) Black Tartary	92·2	80·9	91·7	63·5	0	83·9	85·2	76·5	86·1	90·4	71·0	88·8	79·0
(12) Yielder	89·6	93·0	73·0	73·9	0	95·2	91·3	87·4	86·1	86·5	68·0	85·0	87·2
(13) Black Winter	83·9	—	88·3	83·5	45·2	87·4	81·7	76·1	86·5	86·5	77·1	85·1	83·4*
(14) Marvellous	85·2	—	80·4	58·7	0	89·6	80·4	83·5	83·5	86·5	65·9	82·4	79·6*

A. = autumn sown, S. = spring sown. * 4 years only.

surviving plants is actually greater in the autumn sowings than in the corresponding spring sowings. With one small exception this is a feature common to all the varieties included in this series of tests. The winter of 1928–9 was unusually severe, and cold, dry desiccating winds persisted well into March; even then the relaxation of the winter conditions was very slow and unaccompanied, as is more usual, by rain, with the result that many plants which were not completely killed by the frost succumbed under conditions amounting to a long extended drought. Only three varieties, Grey Winter, Black Winter, and Avoine d'hiver, an oat of very similar character to the first-named, survived this test.

Several of the hybrid selections were grown in the open in the winter of 1928–9 in such a manner as permitted of germination and survival counts being made. The manner of seeding was identical with that described for the variety tests, but in this case each selection was allocated nine sections of 115 seeds per section, or a possible total of 1035 plants. In the following summary the mean percentage of surviving plants for the nine sections under each selection is given as a percentage of the number of seeds which actually germinated, the final count on which the results are based being made in April, 1929. As some data on early growth habit are discussed later, the classification of each of the selections in respect of this feature is also included:

		Early growth habit	Percentage of surviving plants
Parents	Grey Winter	Prostrate	86·7
	Argentine	Semi-erect	0·0
Selections	105/3	Prostrate	63·8
	105/7	„	57·0
	105/8	„	78·0
	109/1	„	69·4
	109/3	„	64·1
	109/4	Semi-prostrate	31·6
	138/1	Prostrate	65·4
	152/1	„	38·6
	152/2	„	48·3
	152/3	„	52·9

The conclusions which may be drawn from a statistical examination of the results are that:

Grey Winter is superior to all selections except 105/8 at 1 % point.
Grey Winter is superior to 105/8 at 5 % point.
105/8 is superior to all selections except 109/1 at 1 % point.
105/8 is superior to 109/1 at 5 % point.
109/1 is superior to all selections except 105/3, 109/3 and 138/1 at 5 % point.
105/3, 109/3 and 138/1 are superior to 152/1, 152/2 and 152/3 and 109/4 at 1 % point.
105/3, 109/3 and 138/1 are superior to 105/7 at 5 % point.
105/7 is superior to 109/4, 152/1 and 152/2 at 1 % point.
152/2 and 152/3 are superior to 152/1 and 109/4 at 1 % point.
152/1 is superior to 109/4 at 5 % point.

An outstanding feature of the observations is the wide difference existing between the two parents; next it will be noted that no selection of *any* family equals Grey Winter in the character under review, although 105/8 is a very near approximation thereto. The selections form a series of gradations of value between the parent with a high survival rate at one extreme, and the parent exhibiting no resistance at the other. Finally, it may be emphasised that there are wide and significant differences between individual members of families. A case in point is 109/4 which is significantly inferior to 109/1 and 109/3.

In considering these results it must be borne in mind that the survival of a plant does not imply the immunity of that plant to frost damage; the survival rate is indeed an extreme measure comprehending within its numbers a full range from damage-free plants to those which survived by a small margin only. As a whole the results provide evidence of segregation of the physiological conditions of resistance and non-resistance to cold, but the very many degrees of resistance imply a complex manner of segregation.

The observations on the winter hardiness of some of these hybrids and certain other varieties have been pursued a step further by a series of freezing trials and field observations conducted by the courtesy of the Sveriges Utsädesförening, at Svalöf, Sweden, under the direction of Prof. Å. Åkerman. Before commenting on the results obtained there it is necessary to describe very briefly the origin of a series of oats which are at present denominated the "*ex* 109/1 series". The selection 109/1 is one of three members of the family 109, one of the white-grained segregates included in the summary just considered. A small number of grey-grained plants were observed at harvest time, 1928, in a plot of this selection. These plants somewhat resembled Grey Winter in features other than the grain colour, but there were, nevertheless, certain features which could hardly be regarded as even wide fluctuations of that variety. The nine aberrant plants were accordingly propagated as single plant cultures in 1929, when six of them exhibited a segregation into grey-grained and white-grained plants. Both series of segregates in the case of the six plants producing such, and the produce of the non-segregating plants maintained a dark green colour throughout the winter, and suffered practically no winter damage of any description. Throughout the following summer they were characterised by strong, upstanding but not coarse straw, and produced grain of high quality. Further single plant selections were made from the most promising culture, No. 1, and these have been increased during the intervening seasons and down to the present time.

Since their isolation there has been no severe winter test, but the various selections have maintained the same, or in some cases a slightly better, winter appearance than Grey Winter. Their grain-yielding capacity, judging by small-scale experiment results, is good. Four of these selections, Nos. 1, 5, 6 and 7, were included in the tests at Svalöf.

Of the other numbered varieties, 125/3, 116/1 and 136/17[1] have not been mentioned before; they are all selections from Argentine × Grey Winter, and each is included for some special individual reason. Thus, 125/3 is one of the few forms exhibiting an erect early growth habit and a fair degree of winter resistance in 1928–9, whilst 116/1 and 136/17 both displayed less resistance under the same conditions. The former, however, was specially characterised by long, resilient straw, and the latter by short and very fine straw which has proved remarkably resistant to "lodging".

As to the nature of the tests: these consisted of freezing trials conducted in the freezing chamber at the Svalöf Institute, and of field observations made on the Institute grounds at the same place. So far as the freezing trials are concerned, it will be observed from Mather and Andersson's (8) account that they were carried out in two seasons under regulated temperatures of $-8°$, $-9°$, $-10°$, $-11°$, $-12°$ and $-13°$ C. After exposure to these temperatures the injury due to freezing was estimated on a scale of grading of 1 to 5, class 1 representing plants that were completely killed, and class 5 those uninjured, the intermediate values representing intermediate amounts of damage. By adopting 10 as an average figure for the whole series of freezing tests, the individual values finally arrived at were as shown in Table VIII, which also includes the percentage of plants surviving when grown in the open (Mather and Andersson (8)).

The freezing tests and the open growth trials at Svalöf show a large measure of agreement, for it will be observed that with few exceptions only those named varieties and selections exhibiting the average figure 10 in the freezing tests gave any surviving plants in the open, whilst those exceeding the average produced the largest number of surviving plants under field conditions.

In general these findings are in accord with the results obtained at Cambridge in the winter 1928–9, but in specific cases differences may be observed which indicate the necessity for further trials. In the artificial freezing tests Grey Winter is above the average value for the whole series, and the named varieties such as Abundance, Bountiful and

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[1] Recently introduced into general use under the name "Resistance'.

Marvellous (to which in this respect Argentine may be likened) fall considerably below it. The numbered hybrids 109/1/5 and 109/3/8 are graded similarly to Grey Winter, and 105/8 below it, with 125/3, 116/1 and 136/17 inferior in the order now shown. The *ex* 109/1 series is markedly superior to Grey Winter.

Table VIII.

		Percentage survival	
	Freezing figure	Sweden 1933–4	England, 1928–9
Grey Winter	11·4	12·7	87·0
	10·8	18·7	—
Marvellous	8·4	0·0	0·0
	7·5	0·0	—
Bountiful	8·6	0·0	0·0
	7·0	1·0	—
Abundance	7·4	0·0	0·0
	7·6	1·0	—
109/3/8	11·1	36·7	69·0
	12·8	62·0	—
109/1/5	11·5	46·7	64·0
Ex 109/1 Cult. 1	13·1	63·0	—
Ex 109/1 Cult. 5	13·0	55·0	—
Ex 109/1 Cult. 6	13·1	46·7	—
Ex 109/1 Cult. 7	12·3	47·7	—
125/3	10·4	11·3	Fair
105/8	10·8	23·9	78·0
116/1	9·3	9·0	—
136/17	7·7	0·7	Poor
136/17	7·7	1·0	—
136/17	8·3	0·3	—
S. 81	10·2	19·0	—
S. 82	10·8	41·0	—
1/2/3/54	9·2	28·0	—

The observations made in the field at Svalöf are more difficult to reconcile with those obtained at Cambridge, for at Svalöf the three hybrids 105/8, 109/1/5 and 109/3/8 were all distinctly superior to Grey Winter. The *ex* 109/1 series again prove the most resistant selections, and as a series are in advance of everything else included in these tests. 136/17 shows no resistance in the field and is the least valuable of all the hybrids in this respect.

(2) *Straw.*

Secondary only to a high degree of winter hardiness in the combination of objectives to which these investigations are directed is good standing straw. Although the standing power of cereals has formed the subject of numerous researches directed to the problem from many angles, the results have not succeeded in delimiting the characters of a good standing straw to any particular feature or series of features, whether anatomical, morphological, or purely mechanical (Biffen and Engledow (1), Brady (2), Williams (9), Zade (12).

The hybrid progeny of Argentine × Grey Winter exhibited a very diversified range of characters of straw. Evidence of transgressive segregation in length is furnished by the two families Nos. 136 and 147, the former being much shorter and the latter longer than either parent. Between these extremes there was a long series of forms of intermediate lengths, and of different expressions of "fineness". But "fine" and "coarse" straw was not associated with any definite length of straw. Judged purely on standing power, the best straw was that of 109/1, 109/3 and 136/17; in the case of the first two the straw was only 5–8 cm. shorter than Grey Winter, but somewhat coarser than in that variety, whilst in the latter it was at least the same number of centimetres shorter than Argentine and therefore considerably shorter than Grey Winter. Nevertheless the straw of 136/17 is finer than in 109/1 and 109/3. Of all the various selections of the cross that have been under observation, 136/17 is remarkable for its good standing power.

In the case of families 105 and 152 the straw was somewhat longer and less fine than 136/17, but none of the selections of either family exhibited the standing power of 109/1, 109/2 and 136/17.

(3) *Grain quality.*

As in the case of straw characters, so in the physical features of the grain, the various hybrid selections furnished a very diversified picture. Taking the length of the grain first, one extreme was presented by 109/1, which is longer than either Grey Winter or Argentine, whilst 124/3 is shorter and distinctly more spindle-shaped than either parent. Between these two extremes there was again a long range of forms of intermediate values.

Table IX. *Showing the percentages of husk in selections of four hybrid families*, 1927–32.

Family No.	Selection No.	1927	1928	1929	1930	1931	1932	Average
67	1	19·7	21·7	20·4	22·0	23·7	21·5	21·5
	5	23·6	24·1	23·4	24·9	25·1	25·7	24·5
	6	21·3	22·6	21·4	22·2	23·9	22·4	22·3
109	1	20·0	20·2	20·6	20·3	20·8	20·6	20·4
	3	20·6	21·0	21·6	22·1	21·5	21·5	21·4
	4	23·0	23·0	22·6	23·0	21·4	21·9	22·5
136	17	25·9	28·5	26·6	29·0	27·3	27·2	27·4
226	2	22·6	23·1	25·0	24·0	22·8	23·3	23·5
	4	23·0	23·0	25·7	23·0	24·5	25·5	24·1

Table X. *Showing the* 1000-*grain weight* (*gm.*) *of selections of four hybrid families*, 1927–32.

Family No.	Selection No.	1927	1928	1929	1930	1931	1932	Average
67	1	43·5	35·6	39·8	34·4	35·9	38·1	37·9
	5	40·4	33·2	40·4	33·5	35·8	35·4	36·5
	6	45·0	34·7	40·8	36·7	38·0	43·5	39·8
109	1	44·8	41·0	40·6	42·7	41·2	40·6	41·8
	3	42·5	40·1	38·2	39·3	37·6	38·5	39·4
	4	45·0	41·7	38·9	40·10	44·5	—	42·0
136	17	39·4	33·6	34·5	34·2	34·5	32·8	34·8
226	2	45·4	39·0	38·9	40·4	42·1	42·0	41·3
	4	43·9	38·7	37·9	41·8	42·4	37·0	40·3

In Table IX the percentages of husk for selections of four families, and in each case for a period of six successive seasons, 1927–32, are shown. In the case of family 109 it will be observed that of the three selections under trial two exhibit a husk content closely similar to Grey Winter, whilst the third is somewhat higher than that parent; 136/17, on the other hand, exhibits a distinctly higher percentage of husk and approximates to the Argentine parent. Families 67 and 226 are both spring sorts, *i.e.* they have not shown any resistance to low temperatures. In the case of family 67, nevertheless, two selections are similar in husk content to Grey Winter, whilst the third, No. 5, shows a consistently higher figure. There is thus no reason to postulate winter hardiness and low husk as invariably associated characters.

Viewing the whole of the selections made in 1926 from the F_3 generation, the husk figures exhibit a complete series of gradations between the two parents, but amongst the units comprising each family there is evidence of small but nevertheless constant differentiation. It will be observed, however, that the differentiation existing within families in no case partakes of the wider range which distinguishes families with a high husk from those with a low husk content.

Proceeding to the 1000-grain weights (Table X), families 109, 226 and 136 provide evidence of transgressive segregation, the first two in a plus and the third in a minus direction.

Regarding the relation of straw of good standing power to the quality of the grain: in view of the generally high husk content of many of the modern varieties with very stiff straw there is a tendency to regard the two characters as being closely associated. This conclusion receives some support in the present investigations from the results obtained with 136/17, which of all the selections made amongst the progeny of Grey Winter × Argentine exhibits the best standing straw, and concurrently a relatively high husk content. At the same time it will be recollected that many "land varieties" are characterised by very high husk content and straw of poor standing power. Moreover, varieties such as Abundance and Victory, which on the average have only approximately 25–26 per cent. of husk, are characterised by good straw. To claim that 109/1 or 109/3 is equal to Abundance and Victory in standing power is probably an overstatement, but the results obtained in a period of six years indicate that these new hybrids are superior in this feature to Grey Winter, and yet do not exceed that variety in husk content, and are superior to it in grain size. Thus, it may be concluded that whilst high husk content is by no means a concomitant of good standing straw, neither is low husk content an invariable index of poor standing straw, and, finally, that a high degree of resistance to low temperatures may be found in conjunction with good standing straw.

The comparisons of Grey Winter and two of the hybrid selections 109/3 and 136/17 may be advanced a further stage by a series of chemical analyses of the whole grain made in the seasons 1931, 1932 and 1933. Table XI shows the average results of these determinations.

Table XI.

	Protein %	Ether extract %	N-free extract %	Crude fibre %	Ash %	Husk % of whole grain	1000-corn weight gm.
Grey Winter	9·39	7·66	69·27	10·02	3·54	21·3	35·5
136/17	8·44	6·89	68·50	12·83	3·31	27·9	33·3
109/3	9·20	7·99	69·34	9·89	3·64	20·6	38·8

It will be seen that in all respects 109/3 is extraordinarily similar to Grey Winter; 136/17 reflects the higher husk content of its grain in the amount of crude fibre, and is, moreover, inferior to the other two varieties in both protein and ether extract.

(4) *Habits of early growth.*

As already noted, the parents Argentine and Grey Winter are strongly differentiated in their respective early growth characters, the former being semi-erect, and the latter prostrate. The habits of growth of the progeny of Argentine × Grey Winter was determined, first, in the F_3 generation, 1926, with the following result. The denominations used in this and subsequent classifications are (1) erect, (2) semi-erect, (3) semi-prostrate and (4) prostrate. In (1) the main stem and tillers from the time of appearing above ground assume and maintain through growth a position at right angles to the surface of the soil, whilst in (4) for a time they assume a definitely horizontal position. In (2) the main stem and tillers assume a position of approximately 35–45° from the vertical, and in (3) 25–35° from the horizontal.

Number of families in each group.

Erect	Semi-erect	Semi-prostrate	Prostrate
6	84	110	36

When subdivided in accordance with their segregation into white-grained, grey-grained and heterozygous grey-grained forms the following result was obtained:

	Erect	Semi-erect	Semi-prostrate	Prostrate
White-grained forms	4	35	19	8
Grey-grained forms	1	13	23	9
Heterozygous grey-grained forms	1	36	68	19

In 1927, that is in the F_4 generation, the habit of early growth of 350 selections from 59 white-grained families was determined with the following result. The early growth form and number of the F_3 parents from which the selections were made in each case is indicated in the left-hand column:

	Erect	Semi-erect	Semi-prostrate	Prostrate	Total
Erect (3)	8	6	—	—	14
Semi-erect (34)	6	151	31	—	188
Semi-prostrate (13)	—	11	76	10	97
Prostrate (8)	—	—	—	51	51
Total	14	168	107	61	

The outstanding features of these figures are the appearance of forms with the erect growth habit, which is exemplified in neither parent, the continuance of the prostrate habit of the F_3 generation unaltered in the F_4 generation, and the segregation observed in those families which were designated semi-erect and semi-prostrate in the F_3 generation. In the case of the former the range extended from erect to semi-prostrate, and in the latter from semi-erect to prostrate. Both the semi-erect and the semi-prostrate habits thus exhibit the features of hybrids.

The possible significance of the different early growth habits in their bearing on the present investigation may be considered under several heads, but the primarily important relationships are those existing between early growth habit and resistance to winter conditions, strength of straw, and quality of the grain.

It is a significant fact that the hybrid selections exhibiting a high degree of winter resistance (p. 429 and Table VIII) are all either prostrate or semi-prostrate in early growth habit; moreover, it has been noted throughout the period of these investigations that forms with these early growth characteristics are further characterised by the narrower, darker green leaves so typical of Grey Winter and Black Winter in their early stages of growth. Nevertheless, there can be little doubt that 109/4 and 136/17, both of which are semi-prostrate, possess very low degrees of resistance to winter conditions. Further, 125/3 is an outstanding example of the erect habit of growth associated with a "fair" degree of hardiness. Thus, although the highest degrees of winter resistance have been found in forms exhibiting prostrate or semi-prostrate habits, all forms with these habits are not highly resistant.

From observations made on the progenies of the hybrid under immediate review and on many other named varieties, high tillering capacity and the prostrate habit of growth are closely associated. The hybrid selections 109/1, 109/3 and 136/17 are high tillering forms, the two former being prostrate and the latter semi-prostrate in early growth; they are all, in addition, characterised by good standing straw. Many of the semi-erect and erect forms are equally good in this respect. But in both divisions of early growth habits, forms with less desirable straw have been found. Thus, the conclusion finally arrived at is that there is no close association between any definite early growth habit and the standing power of the straw.

From a survey of the named varieties in general use it may be inferred that forms with erect and semi-erect early growth habit and stiff, upstanding straw produce grain with a high husk content. Such a generalisation requires modification in the case of varieties such as Abundance, Victory, etc., which, whilst erect in early growth habit and characterised by strong straw, produce grain with a husk content of an intermediate value. At the same time the named winter-hardy varieties usually produce grain of high quality, *i.e.* low husk, and they are prostrate or semi-prostrate in early growth. Hence there has arisen a natural tendency to associate the prevailing spring varieties and the concomitant erect or semi-erect early growth habit with a medium to low quality of grain, and the winter varieties with their prostrate and semi-prostrate habits with a higher grain quality. But amongst named varieties there are a few with unquestionably spring habits, for example, Gris de Houdan (*vide* Table V), which produce grain of high quality; and amongst hybrid selections 67/1 (Table IX) is a notable example of the same combination of characters. In the case of Gris de Houdan, moreover, the early growth habit is prostrate, whilst in 67/1 it is semi-erect.

The observations made on various selections *ex* the cross Argentine × Grey Winter during a number of seasons indicate that there is no very close relation between the husk content and the early growth habit. Thus:

	Early growth habit			
	Erect	Semi-erect	Semi-prostrate	Prostrate
Number of selections under observation	5	24	5	11
Range of percentage of husk in whole grain amongst selections	22·9–25·0	20·9–26·3	22·8–27·7	20·4–26·0

The number of selections in each class of growth habit is admittedly small, but with this qualification the evidence is in line with the conclusion just stated.

(5) *Earliness in ripening.*

It has been shown that in their ability to resist conditions of low temperature, whether artificially induced or in open field conditions, the selections 109/1 and 109/3 are very similar to Grey Winter, and in some circumstances may, indeed, exceed that variety. A feature in which they exhibit an unquestionable superiority is that of earliness in ripening, for in this respect they have throughout a period of seven seasons been earlier in ripening than the same parent by from 7 to 10 days. Apart from its agricultural value, which is considerable, greater earliness in ripening is interesting in the present case in so far as it indicates the possibility of securing a physiological character actually associated with spring forms in varieties possessing winter-resistant characteristics.

True winter forms of cereals have been defined as those which, being sown out in spring, give rise only to tufts of radical leaves, continue tillering during the whole summer and at last die without having produced fruit-bearing stems (Malzew (7), p. 479), or, again, as forms which under conditions of spring sowing function as biennials, producing abundant tillers in the first year, and flowering stems in the second (Gassner (5)).

Judged by these definitions, it will be seen from Tables II and IV that Grey Winter and Avoine d'hiver—a French form of that variety—do not afford the same measure of ready classification, for when sown in spring they both produced flowering shoots and grain like the spring varieties with which they were grown in comparison; the only observed difference between the two classes of varieties was that the winter oats were from 5 to 7 days longer in attaining full ripeness.

Whilst it is always difficult to decide within a range of 2 or 3 days when a plant is fully ripe, the determination of the date of emergence of the first spikelet from the leaf-sheath is only a matter of observation, but one, nevertheless, that provides a very close measure of the final relative earliness or lateness in ripening of a variety. In Table XII, therefore, the figures presented show the number of days elapsing between sowing and the emergence of the first spikelet, in the case of the twelve varieties to which reference has been made previously in regard to other features.

Taking the averages of the autumn and spring sowings it will be seen that in each of the five seasons (1928–9 being excepted) the number of days difference between the two is less than the expected difference, by periods varying in different seasons from 6·7 to 13·5 days.

The definiteness of the time of spikelet emergence as a varietal characteristic is attested by the average figures for each variety; it will be observed, for instance, that on this basis Bountiful is the earliest variety in both spring and autumn sowings, and that Hopetoun, Sandy, Potato and Black Tartary are consistently late. Contrary to expectations Grey Winter occupies an intermediate position in both sowings; it was, however, the last variety to attain full ripeness by from 5 to 7 days, which indicates a relative lag in this variety between the time of spikelet emergence and that of full ripeness. The selections 109/1 and 109/3 when autumn sown throughout an experimental period extending over six years have shown a spikelet emergence 5–7 days ahead of Grey Winter and have ripened 7–10 days earlier than that variety. Thus, these two selections neither actually nor relatively differ from spring varieties in these particular features.

Although the data do not extend over the same number of seasons, it may be noted in this connection that the results obtained with the *ex* 109/1 series, are very similar to those obtained with 109/1 and 109/3. And since at least one of the selections of the *ex* 109/1 series appears to be definitely more resistant to low temperatures and to winter conditions than Grey Winter, the result emphasises the possibility of the association of relative earliness in ripening and high degrees of winter hardiness.

Finally, such of the results presented above as deal with resistance to winter conditions demonstrate the existence of different degrees of this character, and the term winter hardy when applied to varieties assumes

Table XII. *Showing the number of days elapsing between sowing and the emergence of the first spikelet*, 1927–32.

Harvest year ...	1927		1928		1929		1930		1931		1932		Average, and order of varieties (in brackets)	
Varieties	Sown 30. xi. 26	Sown 5. iii. 27	Sown 12. xii. 27	Sown 5. iii. 28	Sown 30. xi. 28	Sown 9. iii. 29	Sown 5. xi. 29	Sown 5. iii. 30	Sown 5. xi. 30	Sown 5. iii. 31	Sown 5. xi. 31	Sown 5. iii. 32	Autumn-sown (5 seasons)	Spring-sown (6 seasons)
Grey Winter	191	104	184	109	198	105	218	109	218	107	224	115	207 (7)	108·2 (7)
Avoine d'hiver	190	104	184	108	201	105	216	109	218	107	220	115	205·6 (2 *aeq.*)	108·0 (6)
Gris de Houdan	190	103	183	108	—	104	217	108	218	107	220	113	205·6 (2 *aeq.*)	107·2 (5)
Sandy	195	105	187	111	—	106	220	110	219	107	222	114	208·6 (9)	108·8 (9)
Hopetoun	197	106	187	115	—	111	224	114	224	114	228	119	212·0 (11)	113·2 (12)
Ligowo	192	102	184	109	—	103	217	108	219	104	220	113	206·4 (6)	106·5 (3 *aeq.*)
Abundance	191	102	182	108	—	103	217	106	219	107	220	113	205·8 (4)	106·5 (3 *aeq.*)
Bountiful	183	101	182	106	—	101	214	106	213	102	216	111	201·6 (1)	104·5 (1)
Potato	195	105	191	113	—	110	224	114	221	115	224	117	211·0 (10)	112·3 (11)
Argentine	192	104	185	109	—	103	218	110	221	110	224	116	208·0 (8)	108·7 (8)
Black Tartary	195	105	192	112	—	109	222	114	224	113	230	119	212·6 (12)	112·0 (10)
Yielder	191	102	184	107	—	103	217	107	218	104	220	113	206·0 (5)	106·0 (2)
Average	191·9	103·6	185·4	109·6		105·2	219·0	109·6	219·3	108·0	222·3	114·8	207·5	108·5
Difference	88·3		75·8				109·4		111·3		107·5			
Actual number of days between the dates of autumn and spring sowings	95		84				120		120		121			
No. of days by which the autumn sowings are accelerated	6·7		8·2				10·6		8·7		13·5			

a relative as well as an absolute value. Grey Winter itself may be regarded as exemplifying a relative position only, and a variety possessing greater winter resistance, under the conditions prevailing in this country, might exhibit the features of those varieties which Malzew (7) defines as "true winter forms" or assume the biennial features described by Gassner, *loc. cit.* The condition just described above, however, must also be visualised as a possibility.

(6) *Yield of grain.*

The true value of any variety of crop plant to the producer is measurable by the amount realised for the produce *less* the cost of production. An important item in the cost of producing cereal crops is that of harvesting, and a "lodged" crop may readily entail double the expense in cutting and handling that an upstanding crop does. Thus the value of an additional yield of grain may be wholly absorbed in the extra expense involved in securing a crop. Apart therefore from the grain-yielding potentiality in the case of cereals, the possession of good straw in any variety is an asset of considerable value, nevertheless one that is at the same time difficult to evaluate. The hybrid selections under discussion were all selected, primarily, on the basis of good straw and *ceteris paribus* are superior to Grey Winter in this respect.

Attempts have been made to determine the relative yields of grain of selections of 109 and of 136/17 initially under the Latin square arrangements of small replicated plots, and later in drill-strip tests carried out by the National Institute of Agricultural Botany.

The seasons 1932–3 and 1933–4 were abnormally dry, and the results may probably require careful review in the light of similar determinations made when climatic conditions are more normal for the oat crop. With this qualification, and for immediate purposes, it may be stated that whilst the figures obtained in 1933–4 exhibit no differences between Grey Winter and the selections of 109 and 136/17, those obtained in 1932–3 amounted to 8–10 per cent. in favour of 109, and of 10–15 per cent. in favour of 136/17. In 1931–2 the differences in favour of the hybrids were of a like order.

The results obtained by the National Institute of Agricultural Botany were very similar in direction to those provided by the smaller scale tests, but they too are subject to the qualification arising from abnormal climatic conditions.

SUMMARY.

The original object of the breeding investigations described above was to secure a new variety of oats exhibiting the resistance to winter conditions and the grain quality characteristic of Grey Winter, in combination, if possible, with straw of better standing power.

The characters of the hybrids studied in the course of the investigations were accordingly resistance to winter conditions, standing power of the straw, quality of the grain, and the early growth habit, particularly in relation to these three features.

The nearest approach to a variety of the character visualised was found in two selections, denominated 109/1 and 109/3, obtained from the progeny of the cross, Argentine × Grey Winter. In respect of the character of resistance to winter conditions, both these selections exhibited a near approximation to Grey Winter, with which variety, although they both exhibit a higher 1000-grain weight, they are almost identical in husk content and chemical composition. Both selections are also very similar to Grey Winter in habit of early growth and in tillering capacity; they differ from that parent, however, in the superior standing power of the straw, which is at the same time less "fine" than Grey Winter. Finally, both selections exsert their first spikelet 5–7 days before, and ripen 7–10 days earlier than, Grey Winter.

In the course of the experiments, another selection, 136/17, was noticeable primarily on account of its superior standing straw. This oat, although semi-prostrate in habit during early growth, is shorter in the straw than either parent, and despite a high tillering capacity, it produces a small grain with a husk content more nearly approximating to Argentine than to Grey Winter.

Selections which were almost equal to Grey Winter in resistance to winter conditions were isolated and have been described; these were prostrate in early growth habit, but other selections with the same habit did not exhibit an equal degree of resistance. On the other hand, selections with the erect habit exhibited some degree of resistance. Although distinct differences in resistance may be found amongst forms with the prostrate habit it is concluded that this character must be regarded as a concomitant feature of the most resistant forms.

The determinations made on the husk content of the grain led to the conclusion that on segregation a series of forms exhibiting small individual gradations between the respective values of the two parents is produced. In those cases in which they have been studied up to the present the gradations are consistent in value from year to year. Each of the families studied arose from a single plant selected in the F_3 generation and as families, and in respect of husk content, exhibited a series of separate but not widely differentiated gradations of values between the parents. But the range of segregation amongst the individual selections within families was invariably narrower than that existing between the families with the highest and lowest husk values.

A wide range in length of straw was found in the hybrid progenies, from a length exceeding the longer parent, Grey Winter, to a shortness exceeding that of the shorter parent, Argentine. The "fineness" of straw ranged between that exhibited by the two parents.

The range of early growth habit found in the progeny of the cross was from erect to prostrate. Whilst the erect and prostrate segregates produced progeny exhibiting only erect and prostrate forms respectively, the semi-erect gave rise to forms which ranged from erect to semi-prostrate, and the semi-prostrate to forms which ranged from semi-erect to prostrate.

Grey Winter produced normal grain even when spring sown, and thus does not come within the category of a true winter cereal. Several of the new hybrid oats, whilst exhibiting a degree of resistance to low temperatures, similar to Grey Winter, are definitely earlier in spikelet emergence and in ripening than that variety. It is accordingly concluded that resistance to low temperatures and a relative degree of earliness in ripening are not incompatible features.

As some of the original plants of the *ex* 109/1 series subsequently segregated into white and into grey-grained plants it is suggested that they may have arisen as the result of an out-pollination in which Grey Winter was one parent. The high resistance of these oats to low temperatures is found in association with strong straw, grain of good quality, and relative earliness in ripening.

Determinations of the grain yield of the most promising of the hybrid selections have been made, and the results indicate a significant superiority of those selections to the parent, Grey Winter.

I gladly avail myself of the opportunity of expressing my thanks to Professor Sir Rowland Biffen for his valued advice on many occasions and in various connections, and to my colleagues, Mr F. H. Garner, M.A., and Dr H. G. Sanders, for their kind assistance in the statistical examination of the data presented above.

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(*Received January 1st*, 1935.)

THE EFFECT OF THE GROWTH-PROMOTING, APPETITE-STIMULATING OR "PHYSIN" FACTOR ON THE LIVE-WEIGHT INCREASE OF SWINE.

BY GEORGE DUNLOP, *Ministry of Agriculture Research Scholar.*

(From the Animal Nutrition Research Institute,
University of Cambridge.)

INTRODUCTION.

THE feeding and management of young pigs during the suckling and post-weaning periods have for long been regarded as an art. Certain instances of large litters which have reached the average weight of 50 lb. on weaning at 8 weeks old have been reported in the popular agricultural press and elsewhere. It is true that in these journals many spurious claims of litters having accomplished such rates of growth are put forward by commercial firms as the result of feeding their proprietary pig foods, but, on the other hand, some genuine cases have been reported from reliable sources. On analysis, these gratifying performances are found to be achieved on various rations and systems of management. So far, they are unexplainable on the basis of known essential nutrients in the diet. It would appear nevertheless that some food factor has either been present in appreciable amounts in the diet or has been passed on by the mother via the placenta to exert its maximum effect in stimulating the growth phenomenon in the animal. Management by itself would appear to play only a subsidiary role.

Shortly after commencing our investigations on swine nutrition at the Animal Nutrition Research Institute, Prof. Sir Frederick Gowland Hopkins directed the attention of the writer to the results of certain rat experiments carried out in his laboratory by Dr Mapson. We were kindly allowed by Mapson to scrutinise the data (at that time unpublished) which he had obtained and which plainly demonstrated the existence of a hitherto unknown dietary factor in liver and to a less extent in certain other natural foodstuffs. The function of the substance in the animal economy appears to be stimulation of the chief physiological process, that is to say, it is responsible for an increased rate of growth, especially in the early stages, and improves fecundity and stimulates the secretion of milk in the suckling female. The importance of such a substance in the nutrition of domesticated animals is unquestionable, and the possibility of a deficiency in rations of cereals, extracted oil cakes or even pasture grass merits careful investigation.

The first experiments on the action of this factor when added to the diet of swine commenced at the Animal Nutrition Research Institute at Cambridge three years ago, since when a number of papers by other workers on the properties and identity of the substance have appeared (*vide infra*). A few experiments by the writer had been conducted up till the summer of last year. The investigation as it stands is still far from complete, but since it cannot now be carried further the results obtained have been collected and form the subject of the present communication.

PREVIOUS INVESTIGATIONS.

It has been known for some years that a diet consisting of all the known food nutrients—purified starch, fat and protein, along with the various essential minerals and vitamins—does not provide for maximum growth in vertebrates. In 1926 Osborne and Mendel (17) showed that stock rats fed on a basal ration with caseinogen as protein, starch and salts, yeast and cod-liver oil gave a poor growth response compared with animals which received 40 gm. of fresh lettuce in addition to the basal diet. Three years later McCay, Dilly and Crowell (13) reported experiments in which they fed brook trout small amounts of liver constituting 5 per cent. of the total ration. A marked acceleration in growth was obtained compared with the controls which received none.

An accelerated growth response in rats was obtained by Guha (9) in 1931 through feeding sheep liver, pork liver or certain other dietary articles as supplements to a basal diet which he considered adequate. He was unable to identify the factor with any of the recognised nutrients. In the same year Daggs (4) reported studies on the protein requirements for lactation of female dogs and suggested that liver also contained some lactation-promoting vitamin or hormone.

Mapson in 1932 (14) following extensive studies demonstrated that beef liver contains a factor stimulating lactation, accelerating growth rate and improving fertility in female rats fed a complete synthetic diet. He showed that this was not due to the protein, ash, vitamin B_1 or vitamin B_2 which the liver contained. The term "physin" which he applied to the substance has been adopted in this paper, since its constitution, up to the present time, has not been fully worked out.

The results obtained by Mapson were confirmed by Graham and Griffith (8), who showed that the increase in growth was correlated with increased food intake. They suggested, however, that the factor responsible was the vitamin G (B_2) which the liver contained.

Johnson and Palmer (11) refer to experiments in which Ferrin and MacCarty demonstrated the unusual palatability of liver meal, as well as its ability to make a swine ration palatable that had heretofore resisted all attempts to make it so regardless of all the combinations of supplements used. The inclusion of two parts of this supplement in place of two parts of tankage increased feed consumption by approximately 27 per cent. In their own work Johnson and Palmer found that fresh pork liver increased growth and food consumption compared with control animals on a diet adequate as regards known food constituents. When food consumption was controlled there were no significant differences. Liver meal increased growth rate to a lesser degree and beef muscle meal not at all. Growth rate and food consumption were increased to a similar extent, no matter whether pork liver, beef liver or calf liver were used, the increased growth being due to appetite stimulation.

These findings run contrary to the conclusions of Seigers and Smith (18), who assert that they were able to show by the paired feeding method that rats receiving the liver supplement grew at a faster rate than the controls. Examination of their technique, however, shows that they made no adjustment in the diet of the controls for the calorific value of the liver supplements.

Recent experiments by Japanese workers (16) confirms the findings of Daggs and of Mapson with regard to the lactation-promoting properties of physin. They suggest that the lactation-promoting substance of liver may constitute a new dietary principle which they designate as the "lactation factor" or "factor *L*". The factor was also found to be present in yeast and rice polishings, but in so small an amount that very large quantities of these materials were required to support lactation as has already been observed by other workers.

EXPERIMENT 1.

Fifteen animals from three litters (A, B and C) were used in the first experiment; two animals from litter A, two from litter B and one from litter C were placed on each treatment. They were 6½ weeks old and in good condition when the experiment commenced.

The basal ration fed to the animals was composed of 35 parts barley meal, 25 parts ground oats, 30 parts white sharps, 10 parts extracted soya-bean meal plus a mineral mixture. One group of these animals was fed in addition 4 oz. raw minced ox liver per day, the second group 1 oz.

of meat meal per day, and the third group 1½ oz. extracted soya-bean meal per day. These supplements contained approximately the same amounts of protein.

The individual *ad lib.* feeding method was adopted for each animal until it reached 40 lb. live weight, and during this period a record was kept of the amount of food it consumed. Access to the ration was allowed three times per day. The food was moistened with drinking water to render it more palatable. Fresh drinking water was also available. A portion of each pen was fitted with floor-boards and no litter was allowed.

After reaching 40 lb. the animals were fed on the same plane of nutrition till they reached bacon weights ((5*a*), p. 599). When their live weight reached 100 lb. the liver group had their supplement withdrawn and replaced with meat meal. The data relating to these three periods are given in Table I.

Table I. *Exp.* 1. *Live weights of animals* (*in lb.*).

Litter	Animal no.	Sex	Treatment	Initial weight	Weight when liver animal reached 40 lb.	Gain	Weight 9 weeks after reaching 40 lb.	Weight 6 weeks after reaching 100 lb.
A	129	H.	M. M.	26	33	7	100	149
	128	H.	L.	26	40	14	101½	149
	127	G.	S. B.	25½	34	8½	100½	152
A	132	H.	M. M.	24	40	16	97	148
	130	G.	L.	24½	40	15½	104	147
	124	H.	S. B.	25	42	16½	104	150
B	140	H.	M. M.	23½	34	10½	98	147
	133	H.	L.	25	40	15	97	148
	141	H.	S. B.	24½	34	9½	99½	150
B	134	H.	M. M.	22½	36	13½	100	153
	137	H.	L.	19	40	21	100	151
	138	G.	S. B.	23½	37	13½	100½	153
C	119	G.	M. M.	19½	38	18½	99	148
	118	H.	L.	18½	40	21½	95	149
	115	H.	S. B.	19½	37	17½	100	150

H. = Hog, G. = Gilt.
M. M. = 1 oz. meat meal per day.
L. = 4 oz. raw liver per day.
S. B. = 1½ oz. extracted soya-bean meal per day.

The table shows that, during the *ad lib.* feeding period, the liver group grew at a faster rate compared with either group on the other two treatments.

Only one animal receiving liver (No. 130) failed to respond, but at autopsy twelve mature Ascarides were found in its small intestine. A similar degree of worm infestation was discovered in animal No. 141 at slaughter, and this animal made no gain in weight for 6 weeks after reaching 37 lb. live weight. The intestines of all other animals were clear or contained only one worm. It is thus probable that these helminth parasites were the cause of the failure of the liver animal (No. 130) to show a greater gain than either of its mates. Although this animal was a female we were unable to show in later experiments that physin stimulates growth rate in hogs to a greater extent than in gilts. Bahrs reports that he obtained this difference in the response of the two sexes with rats (1).

In making these gains the liver animals had consumed more food in each case than either of the animals receiving the other supplements. It was thus difficult to decide whether the liver supplements increased the live-weight gains through increasing the food intakes of the animals or by enhancing the net energy value of the food. From 40 lb. live weight till slaughter, however, food intake was controlled in the same manner for each animal, and the data obtained over this period answer these points. Between 40 and 100 lb. live weight, during which time each animal continued to receive its supplement, there is no significant difference between the growth rate of the groups. Similarly, after 100 lb., when the liver supplement was withdrawn and replaced by meat meal, the results do not show any after-effects of the liver treatment on the rate of growth.

It is clear, therefore, that the liver must have acted as a stimulant to the appetite and that the increased growth rate, obtained during the first period, was due to the increased food consumption of the animals, while the net energy value of the ration remained unaffected. These findings are in substantial agreement with those of Johnson and Palmer (11), who obtained similar results with rats and arrived at the same conclusions.

EXPERIMENT 2.

In the previous experiment it was shown that small supplements of liver had greater appetite-stimulating and growth-promoting properties than supplements of meat meal or extracted soya-bean meal when these were fed to supply the same amounts of protein. Under practical conditions, however, fish meal is the common protein food in rations fed to piglings in the early stages of growth. It was thought desirable therefore to determine if the popularity of fish meal as a foodstuff for young pigs could be explained on the basis of its physin content. Information was also required for further work concerning the stage in the growth period at which the liver supplement should be fed to obtain maximum gains. An experiment was designed to answer both these points.

Four sows were served about the same time, and these farrowed healthy litters all born within 4 days of each other. When the piglings were 4 weeks old they were allowed access to a ration consisting of 2 parts flaked maize, 1 part bean meal. At the same time two of the litters were fed in addition 4 oz. raw minced liver per pig per day, while the other two received an equi-caloric supplement of fish meal. After weaning, the litters were split up and the treatments reimposed as follows: certain animals in the two litters receiving liver were selected at random and these continued a further 4 weeks on this treatment while the remainder received an equi-caloric supplement of fish meal. Similarly certain animals in the litters which had received fish meal continued to receive this supplement while the others now received the liver supplement instead. The basal ration used after weaning was that of the previous experiment.

Table II. *Exp.* 2. *Live-weight records of animals* (*in lb.*) (*whole period*).

	Treatment							
	1		2		3		4	
Weight at weaning	Gain	Economy of gain	Gain	Economy of gain	Gain	Economy of gain	Gain	Economy of gain
29–32	22½	2·42	22½	2·44	16	3·08	13½	3·42
	19	2·83	21½	2·50	14*	3·36	20	2·41
	—	—	—	—	14	3·41	16	2·65
25–28½	21½	2·23	18½	2·72	17½	2·56	14½	3·04
	17½	2·67	15½	2·73	15½	2·56	15½	2·75
	—	—	—	—	18	2·30	17	2·80
21–24½	20	2·28	15	2·40	16½*	2·67	16½	2·55
	19½	2·07	13*	2·51	14½*	2·53	18	2·50
	—	—	—	—	11	2·50	15	2·45
Average	**20**	**2·42**	**17·6**	**2·55**	**15·2**	**2·77**	**16·2**	**2·73**

Treatment 1 = liver before and after weaning.
2 = liver before fish meal after weaning.
3 = fish meal before liver after weaning.
4 = fish meal before and after weaning.

* Attendant notes that these animals had been scouring.

The results obtained over the whole period and those relating to the last fortnight are given in Tables II and III respectively. It is necessary to consider both these periods for two reasons. First, a consignment of liver which was used as supplement for groups 1 and 3 was obviously abnormal, a large degree of fatty infiltration being present. This caused an onset of scouring in the liver groups towards the end of the first week, and whilst most animals recovered, three in group 3 experienced intermittent scouring attacks till the end of the experiment. Secondly, at the end of the first week of the trial a few animals were still unaccustomed to the technique of individual crate feeding and were only beginning to consume normal amounts of food. On the other hand, a few piglings had settled down straightaway. On account of these disturbing factors the results for the first week are not trustworthy and should be neglected. This is rendered possible by considering the data after the ninth day, at which time the animals were again weighed.

Table III. *Exp.* 2. *Live-weight records of animals* (*in lb.*) (*last* 14 *days*).

	Treatment							
	1		2		3		4	
Initial weight	Gain	Economy of gain	Gain	Economy of gain	Gain	Economy of gain	Gain	Economy of gain
33–39	15½	2·06	16	2·31	11	2·84	8	3·34
	14½	2·47	15½	2·31	9½*	3·05	14	2·22
	—	—	—	—	10½	3·01	11	2·40
28–32	16½	2·14	12½	2·60	12½	2·24	10½	2·40
	13½	2·29	11	2·45	11	2·45	11½	2·76
	—	—	—	—	14½	2·03	11½	2·56
24–28	14½	2·04	11	2·34	11½*	2·61	11½	2·75
	14	1·95	9½*	1·98	9*	2·63	11	2·56
	—	—	—	—	9	2·10	8	3·12
Average	**14·8**	**2·16**	**12·6**	**2·33**	**11·0**	**2 55**	**10·8**	**2·68**

Treatment 1 = liver before and after weaning.
2 = liver before fish meal after weaning.
3 = fish meal before liver after weaning.
4 = fish meal before and after weaning.

* Attendant notes that these animals had been scouring.

In presenting the results it is necessary to group the animals according to their initial weights, since this is known to affect the magnitude of the gains (2). On adopting this procedure the data in the tables show that the greatest gains resulted in each case from the feeding of the liver supplement during both the pre-weaning and post-weaning stages. The larger animals in group 2 made similar gains to those in group 1, but the smaller animals showed little response to the pre-weaning treatment with doses of liver. Similarly no response was elicited from the group 3 animals during the time they received the liver supplements, as is seen on comparing their gains with the group 4 animals fed fish meal throughout.

The animals during the post-weaning period were individually fed *ad lib.*, and the food consumption data show that the increased growth rate of the liver-fed group animals was associated with increased consumption of food, while each unit of gain was obtained more economically from the food consumed compared with the fish-meal group.

The results indicate that the physin content of liver is greater than that of fish meal. It should therefore be possible to decide from the lay-out of the experiment the approximate time at which physin supplements should be included in the diet to obtain maximum growth. In this experiment it will be seen that the response after commencing feeding at the 70th day of age is negligible. The experiment, however, may not have been continued long enough to demonstrate the effects of the supplements when added at this stage.

EXPERIMENT 3.

The larger gains obtained on adding raw liver instead of fish meal supplements to the piglings' ration in the previous experiment lead one to enquire if liver supplements give rise to increased growth rates when added to rations containing fish meal at the 5–10 per cent. level. This is the usual amount employed in practice. Sixty-six per cent. of yellow maize in the diet of the pigs before weaning had also been shown to provide insufficient vitamin A for growth to bacon weights on the diet we had previously used after weaning (6). The post-weaning diet was therefore reconstructed to meet the daily requirement of the animals for vitamin A irrespective of their store of the vitamin at the beginning of the experiment.

During the pre-weaning period the litters used were fed supplements of different foodstuffs. These supplements were raw liver, fresh young grass and cod-liver oil. Three sows with their 17-day-old litters were confined indoors and each litter received one of the above addenda. A fourth sow with her litter was also penned up to act as a control. Unfortunately some time after being confined the litters receiving the supplements were unable to obtain milk from their dams who refused to suckle satisfactorily. The fourth sow also appeared to resent being kept in confinement, and during the night she broke out of her pen along with her litter. It was found that they had obtained access to both milk and green food, and thereafter it was decided to run the litter as a positive rather than a negative control. Consequently the sow and family were turned out of doors to graze and root.

Before time of weaning it was obvious that only this last litter was being properly nursed by the mother. The other litters suffered from lack of milk and showed poor weaning weights.

At weaning the litters were split up into three groups. One group received 4 oz. per head daily of raw liver, the second 4 oz. per head daily of fresh young grass, while the third group acted as controls.

From the previous experiments with the new dietary factor in liver it appeared that the difference between the treatments in this experiment would again be fairly large. It was also desirable that no break should occur in the progress of the animals through accustoming the piglings to the technique of individual crate feeding as had occurred in the previous experiment. For these reasons the group method of feeding the animals was adopted.

The basal ration was fed to appetite to all three groups twice per day. It was composed of barley meal 40 parts, maize meal 20 parts, weatings 24 parts, extracted soya-bean meal 10 parts, fish meal 6 parts. These foodstuffs were further supplemented with 1 per cent. cod-liver oil, 1 per cent. ground limestone, 0·5 per cent. common salt and 0·06 per cent. ferric oxide.

Table IV. *Exp.* 3. *Live-weight records of animals* (*in lb.*).

	Post-weaning treatment					
	Liver		Green-food		Negative contro	
Litter: pre-weaning treatment	Initial weight	Gain	Initial weight	Gain	Initial weight	Gain
Liver	28½	36	27	21	25	16½
	26	22	28½	29½	24½	23
	25½	29½	21½	21½	24	26½
	—	—	28	13½	—	—
Average		**29·2**		**21·4**		**22·0**
Green food	26½*	26½	20½	22½	16½	18½
	30½	30	23½	24	29	16
	32	35	—	—	29	28½
Average		**30·5**		**23·3**		**21·0**
"Positive control"	35½	31	36	31	33	26
	31½*	26	37½	33½	38	23
	—	—	35*	7	40	22
Average		**28·5**		**23·8†**		**23·7**
Cod-liver oil	23½	29½	21	22	21*	19
	23	22½	23½	20	20	14½
	22	28	—	—	—	—
Average		**26·7**		**21·0**		**16·8**

* These pigs were scouring during the experiment.
† Or 32·2 if last animal is neglected altogether.

The new treatments were continued for one month when the experiment had to be terminated. The pertinent data relating to this period are given in Tables IV and V.

Table V. *Exp.* 3. *Live-weight records of group* (*in lb.*).

	Group 1 Liver	 2 Green food	 3 Negative control
No. of animals	11	11	11
Weight at weaning 30. vii. 34	253·5	252·5	251·5
Initial weight 7. viii. 34	304·5	302·0	300·0
Final weight 4. ix. 34	620·5	547·5	533·5
Gain during preliminary period 30. vii. 34–7. viii. 34	51·0	49·5	48·5
Gain during experimental period 7. viii. 34–4. ix. 34	316·0	245·5	233·5
Percentage increased gain over negative control	35·3	5·1	—
Food consumption (ration alone)	689	639	611
Economy of gain	2·18	2·60	2·61

The tables show that an increased rate of growth was again obtained by adding small supplements of liver to the diet of the animals. Irrespective of the previous treatment the growth rate of all litters in group 1 has been markedly increased when compared with the corresponding litters in the control group. The interesting feature of these gains is their magnitude. The increase in live weight of the liver animals is some 35 per cent. greater than that of the controls. When it is remembered that these controls received a diet adequate as far as our knowledge of essential minerals and vitamins goes, the results would appear to be of some practical value in the nutrition of farm animals. Their significance becomes all the greater when it can be shown that the controls themselves showed a 10 per cent. greater gain than animals of the same breed and age (or *initial live weight*) under average farm conditions in this country (3).

A similar picture is found on comparing the liver group with the group receiving green food. The increased growth rate through feeding the supplement of raw liver is approximately of the same magnitude as before. Of the original positive control litter one animal in the grass-fed group lost weight during the third week and consumed only small quantities of food for some time afterwards. Deleting this animal's record, which appears reasonable in the circumstances, the remaining two animals show gains as great as their *vis-à-vis* on the liver treatment. It appears that some interaction between treatments has taken place, but we can offer no explanation of the phenomenon.

That fresh green grass contains but relatively small amounts of the growth-accelerating (or appetite-stimulating) factor is apparent from a comparison of the grass fed and the control groups. Only in one litter (the original positive control) is the difference in gains sufficient to be considered significant. The differences between the treatments may be considered greater when the food consumption of the two groups is compared. Unfortunately the technique of the group feeding method does not permit a statistical analysis of the "food consumed" data, and consequently we cannot arrive at any figure to indicate the significance of the differences. The differences, however, are greater than the data in Table V would indicate, since some 15 lb. of dry matter should be added to the total food consumed by groups 1 and 2 to allow for the energy value of the supplements. Furthermore, the consumption of grass would materially lower the net energy of the diet of the group 2 animals, just as the ounce of dry matter in the raw-liver supplements would increase it slightly for the group 1 animals.

DISCUSSION OF THE RESULTS.

Apart from the first experiment, in which food intake was controlled, the investigation had not been extended to a study of the effects of physin on older pigs (over 60 lb. live weight). It is obvious, however, that an increase in the appetite of the pig after this stage would be uneconomic in practice. The excess food consumed would be laid down mainly as depot fat (10), which gives rise to an increase in the amount of food consumed per unit of gain and at the same time decreases the quality of the carcase or grade of the pig.

On the other hand, both before and shortly after weaning the live-weight increase of the pig is chiefly composed of water-soaked protein, which as has been previously pointed out contains nine times less energy than an equivalent amount of fat (12). Consequently an enhanced appetite at this stage entails a relatively greater increase in live-weight gains which are, at the same time, obtained with increasing efficiency of food utilisation. Furthermore, as has also been shown previously, an increased gain of 10 lb. at this stage is equivalent to the saving of a fortnight in the time required to fatten the animal for bacon purposes (5*b*). This is in fact what was accomplished by feeding small supplements of liver for one month to group 1 pigs in Exp. 3.

At Reading, Golding and Foot have conducted experiments similar to our own but have used milk supplements in place of the liver we used in our experiments (7). A comparison of the essential data of their investigation with ours throws some interesting light on the value of milk as a physin supplement and corroborates Mapson's findings in this respect.

At Reading the basal ration fed to the animals was similar to our own, containing as it did all the known minerals and vitamins necessary for swine. The growth rate of the pigs was, nevertheless, increased by including 5 per cent. of dried whole milk in the ration and was further increased when 20 per cent. was fed. At this level they obtained a 33 per cent. increase in growth rate over the controls.[1] Unfortunately the dried whole milk was not tested beyond the 20 per cent. level, as it seems probable from Mapson's work (15) that increasing gains would have been obtained till the 40 per cent. level was reached. In his own work on rats Mapson has shown that milk must constitute 40 per cent. of the dry matter of the ration to give the same acceleration in growth as a daily supplement of 0·3 gm. liver, with which he obtained the maximum effect. This shows that the dry matter of raw liver is approximately forty times as rich in physin as dried whole milk. The pertinent results of these investigations are summarised in Table VI.

Table VI. *Résumé of Cambridge and Reading experiments.*

Centre	Supplements*	Initial weight lb.	Duration days	Daily gain lb.	Percentage increased gain due to supplement
Cambridge	3 % fish meal (control)	32	(38 +) 14	0·77	—
	3 % raw liver	32	(38 +) 14	1·06	**37·1**
	Control	27	28	0·76	—
	2 % green food	28	28	0·79	**5·1**
	2 % liver	28	28	1·03	**35·1**
Reading	Control	—	—	0·95	—
	5 % milk	—	—	1·15	**21·1**
	20 % milk	—	—	1·27	**33·7**
	Control	49	63	0·70	—
	10 % whey	50	63	0·80	**14·0**

* Calculated on a dry-matter basis.

In the Reading experiments, supplements of milk further improved the growth rate of pasture-fed pigs. Such a result might be inferred from the performance of the group 1 and the group 2 animals in our last experiment.

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[1] In these experiments it was suggested that the increased growth of the animals was due to the lactic fermentation encouraged by milk sugar having a beneficial action in aiding digestion.

PRACTICAL CONSIDERATIONS.

A point of immediate practical importance which emerges from these experiments is the ultimate proof that suitable substitutes are available to replace milk in the dietary of swine. In practice, the feeding of milk is known to have a beneficial effect on growth rate and general health, and on it the continued success of Danish pig-feeding and rearing is believed to depend. Apologists for the poor results obtained on many farms in this country explain the slow growth and unthrifty appearance of the animals by pointing out that milk does not form part of the dietary. Apparently the idea that milk might be substituted by some other foodstuff, or perhaps replaced by small supplements of some substance to give even more satisfactory results, has not been entertained.

Unfortunately the investigation could not be extended to examine other foodstuffs, which we intended to test, for the physin factor. The supplement liver which was found to be extremely potent may be considered uneconomic to feed on the farm. In these experiments, although no financial statement is available, the liver animals undoubtedly proved the most economic baconers from the monetary standpoint, since we were able to purchase the liver throughout the two years of the investigation from a local knackery at the price of three halfpence per lb. The total amount of the dry matter of raw liver consumed by each animal did not exceed 1½ lb., and this amount was sufficient to increase growth rate by almost 40 per cent. The source from which the supply was obtained, however, would appear to be limited.

Some other foodstuffs have been shown to contain small amounts of physin. From the results of these experiments and other investigations the following list may be drawn up which will be of interest to the practical expert: liver > liver meal > dried whole milk > whey > green food > fish meal > meat meal or extracted soya-bean meal.

SUMMARY.

Attention has been drawn to the remarkable growth rates obtained in certain litters of pigs under farm conditions.

It has been pointed out that no explanation has been put forward to account for their occurrence.

The literature relating to the new dietary factor, physin (Mapson), essential for optimum growth and reproduction in experimental rats, trout and dogs, has been reviewed.

Experiments are reported in which rations, adequate as far as our present knowledge of essential dietary constituents goes, were fed to young growing swine. In each of three experiments, animals which received in addition 4 oz. of raw minced liver per day showed a 40 per cent. increase in growth rate compared with the controls.

When food intake was controlled according to the new method of individual feeding, the liver animals showed the same gains in weight as the animals receiving the basal diet alone.

Animals receiving a small supplement of liver to their diet showed markedly increased gains over comparable animals fed an equi-caloric supplement of fish meal.

Experiments conducted to determine the effect of feeding physin in the pre-weaning stages were unsuccessful.

When raw liver or young green grass were added as supplements to an adequate diet containing fish meal the animals showed an increased growth rate compared with the controls. The increased gain of the liver animals was seven times greater than that of the grass-fed animals over the controls.

The results are correlated with recent experiments carried out at Reading in which the feeding of whole-milk supplements to adequate diets was shown to give rise to an increased growth rate of the same order as obtained in these experiments. It is suggested that the beneficial effect of milk on growth rate is primarily due to its physin content.

The practical application of the results is briefly discussed and a number of foodstuffs arranged in order of their physin potency.

CONCLUSIONS.

The growth-promoting or appetite-stimulating factor, physin (Mapson), which has been shown to give rise to an accelerated growth rate in laboratory animals, produces a similar marked effect in swine when added to adequate diets of natural foodstuffs. The increased response is approximately 40 per cent.

Most rations for swine in this country are grossly deficient in this factor.

Physin acts through stimulating appetite and general growth. It does not enhance the net energy value of the ration.

The physin content of foodstuffs which may be used to supplement the diet of domestic animals can be arranged in the following order: liver > liver meal > dried whole milk > whey > green food > fish meal > meat meal or extracted soya-bean meal.

The writer takes this opportunity of thanking Dr L. W. Mapson, who has allowed him to consult much of his unpublished work. His thanks are also due to Mr E. A. Porter, who was in charge of the experimental animals.

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(*Received April 8th*, 1935.)

VOLUME XXV OCTOBER, 1935 PART 4

THE DIFFERENTIATION OF GRAIN SAMPLES OF CLOSELY RELATED VARIETIES OF WHEAT BY MEANS OF A SIMPLE MECHANICAL TEST FOR GRAIN QUALITY.

BY O. H. FRANKEL.

(*Wheat Research Institute, Christchurch, New Zealand.*)

THE differentiation of the grain of different varieties of wheat often presents considerable difficulties. In a recent publication, Hudson (1934) supplied a key for the identification of the grain of English wheats, using shape, ridge at the tip, brush, position of the embryo, character of the endosperm, and colour of the grain as diagnostic characteristics. It will be recognised that for samples grown under different environmental conditions a number of these characteristics will lose much of their diagnostic value. Truly reliable differentiation by means of an inspection of the grain, often becomes increasingly difficult with closer relationship of the varieties concerned; and it may become impracticable among lines extracted from one and the same cross, or between hybrid lines and one of their parents.

It should be realised that the power to distinguish the grain of related varieties may be of considerable economic importance, not so much in the distribution of seed, as in that of wheat for milling purposes. Wheat grown and sold for seed nowadays usually is grown under the supervision of some reliable authority, either certifying bodies such as Government Departments, rural societies, seed growers' associations, etc., or reputed seed firms, or the grower himself possesses such standing in the community that his knowledge and experience vouchsafe the production of reliable seed.

Under every one of these conditions the customary field inspection of the crop will insure its varietal trueness with a higher degree of reliability than any inspection of the grain could hope to achieve. In the milling trade, on the other hand, in the large majority of cases grain is bought on the specifications of a grain sample of the line, without any knowledge on the buyer's part of the crop from which the grain was reaped. Accurate varietal differentiation is not important in the grain trade so long as the grading system is based on well-marked varietal or physical properties, such as grain colour and size, moisture, protein content, or bushel weight. But a new situation arises when a special grade is formed for a variety of acknowledged superior grain quality, but with an appearance of the grain which by no means is easily distinguished from that of lower quality varieties. This case will arise frequently where the combination of high yield and baking quality is a major object of wheat breeding policy, *i.e.* in those countries which in the past have mainly relied on imported grain of highest quality to improve their own low-quality product, but now endeavour to free themselves from this necessity by raising the standard of their own wheats. In the majority of cases high-quality forms are crossed with high-yielding standard varieties, and selection aims at a close similarity in type with the latter, combined with some of the grain properties distinguishing the former. Frequently the improvement in gluten quantity or quality cannot be seen on inspection, although readily ascertainable by baking tests. Whilst millers may be prepared to offer special advantages to growers of the improved variety, the difficulty of reliable identification will be irksome to the miller wanting to distinguish the high-quality variety, and to the farmer wishing to establish the identity of his product.

This case has arisen in New Zealand with the production by the Wheat Research Institute of Cross 7, a variety essentially similar to its high-yielding parent, viz. Solid-Straw Tuscan, but markedly superior in flour extraction and baking quality (Frankel, 1934). Grain samples from Tuscan and Cross 7 grown under identical conditions can frequently be distinguished by a slight difference in colour and translucency; but no reliability appears to attach to a visual distinction of samples derived from different environments. In view of the fact that Cross 7 will, for some time to come, command a premium of 2*d.* per bushel above the price for Tuscan, a reliable differentiation of the two varieties is of considerable importance.

During the years of testing, Cross 7 has been grown in a large number of replicated yield trials side by side with Tuscan. Grain samples from all these trials were regularly tested for grain quality, by baking tests, fermentation tests (Pelshenke, 1930) and distensometer tests (Engledow, 1928). The following table contains the results of quality tests from 51 variety trials. In comparison with Tuscan grown under identical conditions, in the baking test Cross 7 is usually, but not always markedly stronger; consequently the baking test cannot serve as a reliable method of identification. The same is true, though to a lesser extent, of the

Quality tests on Cross 7 *and Tuscan samples from replicated variety trials* 1930–1 *to* 1934–5.

		Baking score* (40 = very good)		Fermentation test (min.)		Distensometer test (class)	
Season	Locality	Cross 7	Tuscan	Cross 7	Tuscan	Cross 7	Tuscan
1930–1	Lincoln	32·0	24·5	—	—	—	—
1931–2	Darfield	34·0	23·0	75	54	—	—
	Lincoln	34·5	42·5	139	70	Considerably stronger	Considerably weaker
1932–3	Glasnevin	38·5	37·5	—	—	"	"
	Rangiora	35·5	23·5	—	—	"	"
	Darfield	38·5	28·0	—	—	"	"
	Lincoln	37·0	31·5	100	50	"	"
	Wakanui	35·0	21·0	—	—	"	"
	Timaru	32·5	26·0	—	—	"	"
1933–4	Tuamarina	17·0	11·0	58	30	3	1
	Omaka	26·0	16·0	80	51	3	1
	Cheviot	28·0	18·0	63	51	4	1
	Culverden	34·0	30·0	95	56	3 –	1
	Glasnevin	28·0	27·0	91	54	4	1
	Amberley	19·0	15·0	72	59	3	1
	Rangiora	40·0	25·0	60	35	3	1
	Fernside	33·0	28·0	56	39	3	1
	Springbank	27·0	33·0	60	46	3	1
	Oxford	37·0	27·0	65	43	3	1
	Darfield	26·0	21·0	45	48	3	1
	Hororata	32·0	22·0	57	49	4	1
	Prebbleton	25·0	20·0	52	30	3	1
	Lincoln	26·0	17·0	61	39	3	1
	Irwell	32·0	21·0	51	29	3	1
	Dorie	35·0	37·0	67	47	3	1
	Rakaia	31·0	28·0	78	55	3	1
	Wakanui	34·0	18·0	54	38	3 –	1
	Methven	34·0	26·0	61	46	3	1
	Hilton	32·0	24·0	55	43	4	1
	St Andrews	26·0	27·0	54	32	4	1
	Hadlow	30·0	25·0	57	46	4 –	1
	Morven	28·0	34·0	86	44	4	1
	Pukeuri (A)	22·0	21·0	52	46	3	1
	Pukeuri (B)	28·0	28·0	50	35	3	1
	Ngapara	30·0	28·0	73	49	4 –	1
1934–5	Tuamarina	24·0	10·0	65	39	2 +	1
	Grovetown	11·0	10·0	53	26	2	1
	Omaka	22·0	15·0	55	32	3	1
	Cust	10·0	15·0	57	47	2	1
	Oxford	24·0	11·5	63	48	3 –	1
	Darfield	16·0	5·5	82	65	3	1
	Lincoln	21·5	12·0	62	42	3	1
	Leeston	29·0	18·0	67	54	3	1
	Highbank	29·5	13·5	66	65	3 –	1
	Lyndhurst	24·5	7·0	77	53	3	1
	Sherwood	27·0	13·5	89	58	3	1
	Methven	29·5	14·5	63	47	3	1
	Kingsdown	22·5	16·5	57	49	3 –	1
	Papakaio	24·0	24·0	69	55	3	1
	Tokaraki	25·5	32·5	80	46	3	1
	Incholme	32·5	25·5	79	50	3	1

* The baking scores were supplied by Mr H. E. West, Research Chemist, Wheat Research Institute.

fermentation test. In all except one pair Cross 7 is superior. But there are a number of Tuscan samples which are equal or superior to Cross 7 from other localities.

With the distensometer, however, the case was different. The Cross 7 samples were first compared with the Tuscan lines grown under identical conditions. In every case Cross 7 was very much stronger (results for 1933–4 and 1934–5 not stated in the table). Further, all Cross 7 samples were tested against a series of standards raised at Lincoln, the grouping in "distensometer classes" being the following:

Class	Strength of test sample in relation to standard
1	Equal to, or weaker than Tuscan
2	Stronger than Tuscan, but weaker than ½ Tuscan + ½ Marquis
3	Equal to, or stronger than ½ Tuscan + ½ Marquis, but weaker than Marquis
4	Equal to, or stronger than Marquis

It must be emphasised that the differences between any two distensometer classes are so well marked that differences between 3 and 1, or even 2 and 1, are highly characteristic. It will be seen from the table that Cross 7 in all except three cases was superior to Tuscan by at least two classes, and in the three exceptional cases by one class.

In the seasons 1933–4 and 1934–5, all the Cross 7 samples with lower baking scores than any Tuscan samples were systematically tested against all the stronger Tuscan lines. The result was the same as that in the two previous series of distensometer tests: with one exception (see below) Cross 7 was consistently and markedly stronger. For example, a Tuscan sample with a baking score of 37 was much weaker on the distensometer than a Cross 7 sample with a baking score of 17. One exception, however, has been encountered in 1934–5: The Cross 7 sample from Cust, with the exceptionally low baking score of 10, was equal or inferior to all the stronger Tuscan samples. An inspection of the grain, in comparison with the Tuscan sample from the same locality, made it appear likely that Tuscan grain had become mixed with Cross 7. But even had this not been the case, the low baking score indicates that the Cross 7 line was exceedingly poor, more likely than not due to severe water-logging during the winter. No doubt this sample could not claim a bonus for grain quality.

It may be mentioned that the same method has been used for the differentiation of two other varieties, viz. Jumbuck and Solid-Straw Velvet, the grain of which under certain environmental conditions is hardly distinguishable, in spite of a marked difference in milling and baking value, and accordingly in price. It has been possible by means of the distensometer test to effect a reliable differentiation.

The technique of the distensometer test is sufficiently simple to recommend it for the purpose of identification. The test requires a small amount of flour—obtainable on a small laboratory mill—and a distensometer outfit, to be worked by two operators. One test takes no more than 45 min. (including milling).

It is not intended to discuss in this connection the interrelations between the results of the three methods of testing for grain quality. It may suffice to express the opinion that the distensometer test possibly estimates some component of gluten quality which in its expression is less dependent on environmental conditions than others, and therefore is essentially a varietal characteristic.

SUMMARY.

1. A reliable identification of grain samples of related wheat varieties of different grain values is of economic importance.

2. The distensometer test distinguishes with a high degree of reliability between Tuscan and Cross 7 grain, whereas eye inspection, baking and fermentation tests cannot be relied upon for differentiation between the two varieties.

3. A testing service for the use of farmers, grain merchants and millers would involve no technical difficulties.

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(*Received July 4th,* 1935.)

ANALYTICAL YIELD INVESTIGATIONS ON NEW ZEALAND WHEAT.

II. FIVE YEARS' ANALYTICAL VARIETY TRIALS.

By O. H. FRANKEL.

(*Wheat Research Institute, Christchurch, New Zealand.*)

(With One Text-figure.)

CONTENTS.

	PAGE
Introduction	466
Material and methods	469
A. Chessboard trials (even spacing)	474
(1) Germination, seedling survival and plant survival	474
(2) Tiller development	477
(3) The mature population	481
B. Spacing trials	488
(1) Germination, plant survival and tiller development	489
(2) The mature population	491
C. Census trials	495
The relations between tillering, head formation and yield. The critical period of tillering	497
Conclusions	505
Acknowledgments	508
References	508

INTRODUCTION.

THE task of wheat growing in New Zealand is limited to providing for the needs of a population of 1½ million. Owing to the long distance from other wheat-growing countries it is considered essential for the Dominion to remain independent in its supply of wheat and flour. A sliding scale duty fixes the price of wheat at a level sufficiently remunerative to the grower to insure the production of approximately the required amount. Since 1927, when this duty was introduced, the area under wheat has varied between 236,000 and 302,000 acres, and the annual production between 6½ and 11 million bushels. The requirements for flour milling, seed and stock food being slightly less than 9 million bushels, shortages due to unfavourable seasons are covered by the carry-over from the previous season, and/or by importation. It is only in seasons of excessive yields that export of wheat becomes necessary. Under the compulsory pooling system in force since 1932–3, losses due to the difference between local and export prices are distributed amongst all the wheat growers in the Dominion.

The New Zealand wheat grower therefore as a rule supplies a local market of limited dimensions. Naturally he wishes to retain this market entirely for himself, but in this aim he can be successful only if flour milled from New Zealand wheat possesses generally the standard of quality required for the production of a high-class bread. At present it is held by many millers and bakers that a certain proportion of strong imported wheat has to be added to the local product, and consequently in recent years about 500,000–750,000 bushels (flour counted as wheat) are imported annually from Australia and, recently, chiefly from Canada, the last named being the principal supplier of imported flour.

The combination of high yield with high baking quality is then an obvious task for the New Zealand wheat breeder. Since in the present circumstances the payment of a premium for quality sufficient to reimburse for considerable losses in yield cannot be anticipated, no improvement in quality would be economically attractive to the grower unless retaining at least the present yield level which, with an average yield per acre of 32 bushels, ranges amongst the highest in the world. Systematic importation during the last 6 years of large numbers of foreign varieties did not produce any high-quality forms of sufficient yield capacity under New Zealand conditions. It was realised that an attempt at reaching this objective by means of cross-breeding would be facilitated by a fuller understanding of the yielding characteristics, the "yield structure" (Heuser, 1930) of the facultative parent[1] forms, and also by the lead such information might afford for the selection in the subsequent hybrid generations. A subsidiary economic advantage may be realised if the results of analytical studies facilitate general conclusions in the fields of the optimal local distribution of existing and of newly-bred varieties, of rate of seeding, manuring, etc.

In these analytical studies, the yield of the individual plant has been resolved into its components, viz. the number of heads, and the yield per single head; the latter again into the number of grains, and the weight of the individual grain, so that ultimately the gross yield was resolved into its four components (Engledow and Wadham, 1923–4). Following the lead afforded by the work of Prof. F. L. Engledow and his collaborators at the Cambridge Plant Breeding Institute, the analysis was not confined to the mature population, but it was attempted to establish connections between the habit and behaviour of the growing plant, and the ultimate product.

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[1] By "facultative parents" are meant any varieties which, in view of their yield or quality properties, may be considered as parents in crossing work.

Besides the merely local aspect the main task of these studies has been to test the applicability of the analytical method to problems of plant breeding and plant production. To this end all the more important varieties cultivated in New Zealand have been included in the scope of this investigation, in order to allow of analogies to be drawn between the local varietal distribution, results from variety trials, and agronomic experience on the one hand, and the intervarietal relations in respect of yield characteristics on the other. Beyond this aspect it was hoped to gain further experience on the general conclusions reached in the Cambridge work, particularly with reference to the relations between developmental characteristics, such as rate of tiller development, and yield. The wide difference in the environmental conditions, and the inclusion of a comprehensive range of forms should provide a good test for the general validity of the principles involved. Two of the three varieties chiefly used at Cambridge were included to facilitate comparisons.

The method applied throughout this investigation was that of intervarietal comparison. The results obtained have therefore a comparative, not an absolute significance. They are illustrative of varietal performance in terms of intervarietal differences.

The results of the first season (1929–30) have been published in detail (Frankel, 1931). Some of the chief data will be summarised below.

Engledow and Ramiah (1930) and, more recently, Smith (unpublished) have set out the problems and objectives of an analysis of yield. The latter author has also reviewed the major part of the literature. The English work on the subject was summarised by the author (Frankel, 1932). The statistical approach to yield analysis, on the basis of Fisher's analysis of variance, has been explored by Smith (unpublished). In recent years co-operative schemes on a national and international basis have raised hopes for a rapid elucidation of the ecological factors determining yield differences. Such work on spring wheats has been done in co-ordinated trials in five European countries (Boekholt, 1933). In Australia various institutions are co-operating to organise an investigation into the yield characteristics of a range of about 100 varieties of wheat over the length and breadth of that continent, with the ultimate aim of studying the genetics of yield factors. It is hoped that in the future co-ordinated work will be possible between New Zealand and Australia.

MATERIAL AND METHODS.

Material. A short description of the types, the economic importance, and of the local distribution of the varieties included is given in Table I. The "high-yielding" varieties—New Zealand and English—are listed, according to their time of maturity, as "early" and "late", and the "high-quality" varieties form a separate group.

It is obvious from an inspection of column (8) that only Tuscan and Hunters cover any considerable areas, all other varieties cultivated in the Dominion merely serve special needs, such as Garnet and Jumbuck as quick-maturing crops for late spring sowing, or Yeoman as a highly tillering form useful for winter feeding under special conditions: or else they are particularly adapted to the local conditions of a limited district, such as Velvet in the Hakateramea Valley, or Dreadnought on the rich downs of North Otago.

Tuscan is the "all-round" variety of New Zealand. It plays the rôle which Squarehead's Master plays in England. It can be grown successfully wherever wheat can be grown, on average land as well as under the extremes of light and dry, or wet and cold. Only on the very best of land, on rich loam in good heart, is Tuscan inferior to the dense-eared varieties, chief amongst which is Hunters. Although scattered crops of Hunters can be found in nearly all counties where wheat is grown, its chief centres of cultivation are limited to well-defined districts with particularly favourable soil conditions. Roughly it can be stated that Hunters is associated with dairying, Tuscan with sheep raising. The question whether the ecological factors "climate" (chiefly wind) or "soil" (and, possibly, management) are mainly responsible for the varietal distribution, is of paramount importance in the general plant-breeding policy associated with wheat. If Tuscan owes its wide distribution mainly to its remarkable resistance to shedding of grain, as has been claimed by many, its use as a parent form should be restricted to breeding work for the districts more exposed to the violent and dry north-westerly winds, since it does not belong to the group of medium to dense-eared wheats with large production per head which are characteristic of all the other countries with highest per-acre yields. Crosses with Hunters—which belongs to that group—or other high producers might appear more promising. If, however, it possesses adaptations, apart from wind resistance, which render it more successful than other varieties over the major portion of the wheat area of New Zealand, its claim for use as the chief "high-yielding" parent form would be established.

Table IA. *List of varieties.*

Variety (1)	Abbreviation (2)	Country of origin (3)	Juvenile growth (4)	Ear type (5)	Baking value (5 high, 1 low) (6)	Wind resistance (5 high, 1 low) (7)	Percentage of New Zealand wheat area* 1932–3 (8)	Soil and climate of the chief areas of cultivation in New Zealand (9)
I. Early varieties								
Tuscan	T.	Western Mediterranean	Erect	Lax	2	5	68	All-round wheat; the sole variety on light and medium land, and in windy localities
Solid-Straw Velvet	S.S.V.	?	Semi-erect	,,	1	3	†	On cold soils in South Otago and Southland
Major	Maj.	Australia	Erect	Fairly dense, clubbed	1	1	‡	Early variety, only in the northern districts of the South Island
Dreadnought	D.	,,	Semi-erect	Dense	1	1	3	On rich land with low to medium rainfall (South Canterbury and North Otago)
Cross 7	Cr. 7	New Zealand	Erect	Lax	3	5	—	Similar to Tuscan. This is a new variety produced by the Wheat Research Institute at present being distributed to farmers (see Frankel, 1934)
II. Late varieties								
Hunters	H.	England	Semi-prostrate	Dense	2–3	1	19	On rich, warm soils, not exposed to the strongest wind, *e.g.* Springs-Ellesmere, Kaiapoi-Rangiora, Waimate
Velvet	V.	,,	,,	,,	2–3	1	1·5	On dry, good land, not exposed to heavy winds, *e.g.* Hakateramea, Ngapara
Yeoman II	Y.	,,	Prostrate	,,	3	1	0·05	—
Squarehead's Master	S.M	,,	Semi-prostrate	,,	?	1	0	—
Victor	Vic.	,,	,,	,,	1	1	0·3	On heaviest land, *e.g.* Temuka, Wakanui
III. High-quality varieties								
Marquis	M.	Canada	Semi-erect	Lax	5	2	0·3	—
Reward	R.	,,	,,	,,	5	2	0	—
Garnet	G.	,,	,,	,,	5	2	1	On good land for late spring sowing
White Fife	W.F.	,,	Semi-prostrate	,,	4	2	0	—
Red Fife	R.F.	,,	Semi-erect	,,	5	2	0	—
Tenmarq	Tq.	U.S.A.	,,	,,	5	?	0	—
Jumbuck	J.	Australia	Erect	,,	5	1	0·3	On good land for late spring sowing

* This column is representative of the chief wheat-growing districts of the Dominion, viz. Canterbury and North Otago, embracing 87 per cent. of the wheat area. For the more scattered wheat areas of the South, and for Marlborough and the North Island, no detailed varietal statistics are available. These percentages are quoted from Hilgendorf (1934).

† Cultivated only in South Otago and Southland, which districts are not included.

‡ Cultivated in Marlborough and Nelson, which districts are not included.

In the writer's opinion, wind is not nearly so great a determining factor as is generally assumed by wheat growers. Losses due to shedding of grain are readily seen—on the ground as well as on the heads—and therefore tend to receive more weight from the observer than a slight difference in tillering or ear size, though in its effect on yield this may heavily outweigh a certain amount of "shaking". There is evidence to show that in circumstances where Hunters is specially successful it outyields Tuscan even in spite of losses by shedding. In a variety trial at Lincoln a strong north-west storm damaged severely the Hunters crop, which, however, still yielded 2·9 bushels per acre more than Tuscan, although the latter had suffered no wind damage whatever. And even in the most exposed districts farmers frequently prefer Hunters to Tuscan. In these analytical yield studies an attempt has been made to assess possible causes, apart from wind resistance, for the respective yield properties of Tuscan and Hunters.

All experiments were conducted at Lincoln on the same field, with the exception of the first season, during which an adjacent field was used. It was realised that the most desirable procedure would be a series of co-ordinated experiments extending over the major wheat-growing districts, analogous to the admirable annual census studies carried out since 1927 by the Department of Agriculture in Victoria (Forster and Vasey, 1931, 1932, 1933). Since, however, the amount of work involved precluded the trials being duplicated in any one season, the Lincoln district has the particular advantage that Tuscan and Hunters—the chief alternative varieties—occupy about equal areas. Further, in variety trials at Lincoln during the last seven years, the two varieties were equal in yield twice, Hunters was superior once and Tuscan four times. In consequence it appears that in this locality the intervarietal differences find a more equitable expression, and that they would become more readily applicable elsewhere, than results obtained in localities of a more extreme nature.

Methods. Three sets of experiments were conducted side by side.

A. "*Chessboard*" *trials*, after Dr E. S. Beaven, described by Engledow and Yule (1927). Hand-sowing with equal spacing, 6 × 2 in.; each plot contained eight rows of twenty-five seeds; outside rows, and three outside plants on both ends of each row were discarded at harvest, as well as all plants not in possession of both neighbours. There were twenty replications. Sowing in 1929 with "Beaven's dibber", since then with "Woodfield dibber"[1]. The rate of seeding employed, corresponding to about 44 lb. per acre, represents only one-half of the customary seeding[2].

B. "*Spacing*" *trials*. Hand-sowing, length of rows 16 ft., 6 in. on both ends discarded; rows 7 in. apart, spacing in the rows ½, 1¼, 2, 4, 12 in. 1929–30 three, 1930–31 four replications. Number of plants per plot see p. 488. One guard row of each separated any two adjacent varieties with spacings equal to those of the adjacent varieties. Four guard rows separated different spacings.

In all hand-sown experiments (groups A and B) at harvest the plants were pulled with their roots and immediately sorted and bundled according to the number of heads, so that the sheaf from every individual plot consisted of a number of small sheaves of plants with 1, 2, 3, ... heads. The number of plants in each bundle was determined at threshing. The grain was weighed after being stored in the laboratory for several weeks after threshing, to equalise moisture content. The weight of 1000 grains was obtained for all tiller classes individually by counting all the grains in fractions of under 20 gm., and a 20-gm. sample in larger ones. To obtain the average weight of 1000 grains for the whole plot, a 50-gm. sample of the mixed grain (from all tiller classes) was counted.

C. "*Drilled*" *trials*. Samples were taken from drilled variety trials following the census method (Engledow, 1926). All samples were two foot-lengths of a coulter row, alternating between the two central coulters of the drill, samples being marked by pegs, 100 samples per variety (twenty in each of five drill-strips). At harvest all the plants of each sample were pulled and their number noted on the label. The number of heads was determined at threshing; the weight of grain following several weeks' storage after threshing. The weight of 1000 grains was obtained in 1932–3 by counting the grains in all the individual samples, in 1933–4 by counting the grains in 50 gm. of the composite samples from each of the five drill-strips (samples 1–20, 21–40, etc.). The statistical examination of A and B followed Engledow and Yule (1927, pp. 17 ff., statistical method for chessboard trials). In the Census trials the analysis of variance was applied for the determination of standard errors.

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[1] The "Woodfield dibber" has been evolved at the Wheat Research Institute by C. V. Woodfield. It has been described in the *First Annual Report* of the Institute, 1931, and further details can be obtained from the author.

[2] Hudson and Stafford (1934) have shown that in variety trials a rate of seeding above the customary one is more likely to approach optimal population density for all varieties, since yields tend to rise with rates of seeding increasing up to 30 lb. and more above the normal rate. The present author agrees, however, with Smith (unpublished) that in analytical trials existing implements and convenience of working plead for a wider spacing, although Smith considers 4-in. distance between rows more desirable. Furthermore, varietal differences are more pronounced in more widely spaced populations. Finally, the crop density and yielding capacity of hand-sowings is superior to that of comparable drilled crops, owing to better seed bed, cultivation, freedom from weeds, etc. (Engledow and Ramiah, 1930) so that a lower rate of seeding in the former is likely to be as effective as a higher in the latter.

The major part of these investigations, extending over five seasons, was conducted on the lines of chessboard trials. As a subsidiary, in view of the differential varietal reaction to varying conditions of spacing demonstrated in the Cambridge experiments (Engledow and Wadham, 1923–4; Engledow, 1925; Engledow and Ramiah, 1930), representatives of various "high-yielding" types were tested under conditions of varying spacing.

Owing to the large variation in spacing existing in field crops as demonstrated by Engledow (1926, 1928) and Doughty and Engledow (1928), high adaptability to varying spacing must appear as a considerable advantage. It was further considered likely that adaptability to spacing might be considered to a certain degree an index for adaptability to varying environmental conditions (Frankel, 1931).

Finally, a comparison of varietal differences under the conditions of hand-sowing and drilling appeared desirable, if only as a check on the validity of the results of hand-sown trials when applied to field conditions.

The chessboard trials extended over five seasons, during the first of which the developmental determinations were omitted. The spacing trials were conducted during the first two, the census trials during the last two seasons. The varietal composition of the trials differs from season to season, but certain "indicator varieties", viz. Tuscan, Hunters and Yeoman, were included in all seasons. Tuscan served as the standard variety, for purposes of comparison, in every trial throughout these experiments, so that when in any season the large number of varieties necessitated the laying down of two or three chessboards, Tuscan was included in each of them.

The aim has been to sow in each year between May 5 and 12. From the following list (p. 474) of sowing dates it will be seen that, owing to unfavourable weather conditions, delays of from 1 to 3 weeks became necessary in three seasons. This must be borne in mind in intervarietal comparisons of developmental characteristics. Census trials in each case were sown on the same day as the corresponding chessboards.

The seed used in these trials had been raised at Lincoln in the preceding year, as far as possible under similar conditions of soil, sowing dates, etc. The necessity for a comparable origin of seed for variety trials has recently been demonstrated by Boekholt (1933).

Sowing dates of analytical yield trials.

	Chessboard	Spacing	Census
1929–30	May 23–24	June 5–6	—
1930–1	May 15 and 19	May 26–28	—
1931–2	May 5	—	—
1932–3	June 2	—	June 2
1933–4	May 11	—	May 11

Crops preceding the experimental plots in each year.

1929–30	Rape
1930–1	Peas
1931–2	Fallow
1932–3	Oats and Italian rye-grass
1933–4	Oats and vetches

Rainfall and temperature during the months between sowing and harvest are recorded in Table IB.

A. CHESSBOARD TRIALS (EVEN SPACING).

(1) *Germination, seedling survival and plant survival.*

Engledow and Ramiah (1930) demonstrated the existence of characteristic intervarietal differences with respect to germinating power. Since the establishment of such differences in germination and plant survival may supply information on the causes of differential yields per acre, the percentages of plants that germinated (5–6 weeks after sowing), and of plants which matured heads were determined. In view of the fact that in the investigation quoted above, the majority of the losses occurred during the first two or three months, *i.e.* in the seedling stage, a further count was conducted at the end of the winter (about 3½ months after sowing, "seedling count") in order to determine which were the actual proportions of losses at various periods of development.

An interseasonal comparison shows that during two seasons (1933–4, and 1932–3 with the exception of two varieties) losses were small, whereas in the first and second seasons they were much heavier. The data for 1930–1 are incomplete and therefore do not allow of a full analysis, but it may be suggested that the drought in the early winter and the excessive rainfall in August may be responsible for the losses before and after the seedling counts. There are no intervarietal differences in mortality. In 1931–2 germination was normal, with an average of 95·3 per cent. But in the subsequent period, the losses in some varieties were very considerable. All varieties suffered except Tuscan—it is noticeable that in none of the three chessboards did this variety incur any losses at all. The chief losses were amongst the late and the high-quality

Table IB. *Temperature and rainfall during growth period at Lincoln, New Zealand.*

Month	1929–30				1930–1				1931–2				1932–3				1933–4			
	Temperature			Rainfall in.	Temperature			Rainfall in.	Temperature			Rainfall in.	Temperature			Rainfall in.	Temperature			Rainfall in.
	Mean	Max.	Min.		Mean	Max.	Min.		Mean	Max.	Min.		Mean	Max.	Min.		Mean	Max.	Min.	
May	44·8	54·0	35·7	0·61	46·5	57·2	35·8	1·81	47·9	57·4	38·4	0·83	47·1	55·3	38·9	2·83	46·5	54·8	38·2	2·23
June	45·8	54·1	37·6	4·33	40·4	50·9	29·9	0·67	41·8	50·6	32·9	3·65	42·9	51·2	34·6	0·79	40·6	47·0	34·3	1·29
July	42·3	48·8	35·8	3·59	39·3	47·6	31·0	1·46	41·2	49·3	33·1	2·01	39·8	48·3	31·4	2·26	42·4	49·1	35·7	2·53
August	43·7	53·0	34·5	1·45	41·1	49·0	33·2	4·94	44·2	51·8	36·7	1·24	42·4	51·1	33·7	1·76	44·3	53·2	35·5	1·03
September	47·9	58·0	37·8	1·68	44·5	52·4	36·6	4·00	45·0	52·8	37·1	2·46	47·4	55·1	40·3	0·95	49·8	58·8	40·9	1·08
October	53·1	63·1	43·1	0·14	48·6	58·5	38·7	1·94	54·5	66·2	42·8	1·87	52·8	60·4	45·2	4·84	54·4	65·5	43·4	1·44
November	55·7	66·1	45·3	0·92	52·4	62·4	42·4	2·61	58·0	70·0	46·1	1·78	57·4	67·9	46·9	1·67	54·5	64·7	44·3	1·36
December	59·0	68·3	49·8	3·92	57·5	68·4	46·6	1·57	60·9	71·2	50·1	0·42	57·9	67·3	48·5	2·18	58·1	67·8	48·5	3·09
January	57·8	66·7	49·0	5·37	60·8	71·8	49·8	1·18	61·6	72·0	51·2	0·47	63·7	74·5	53·0	2·26	59·3	68·6	50·1	3·79

Table II. *Chessboard trials, germination, seedling survival and plant survival.*

		Average number of plants per plot, in percentage of seed sown			Plant survival at harvest, in percentage of seedlings (3½ months)	Losses from sowing to harvest, in percentage of seed sown			
Season	Variety	Germination (5–6 weeks)	Seedling survival (3½ months)	Plant survival (harvest)		Germination (up to 5–6 weeks)	Seedling stage (from 6 weeks to 3½ months)	Maturation (from 3½ months to harvest)	Total (sowing to harvest)
1930–1	I. Early varieties								
	Tuscan	—	88·5	—	—	—	—	—	—
	Solid-Straw Velvet	—	87·7	—	—	—	—	—	—
	Major	—	89·0	—	—	—	—	—	—
	Dreadnought	—	91·9	—	—	—	—	—	—
	Marquis	—	85·5	—	—	—	—	—	—
	S.E.	—	2·20	—	—	—	—	—	—
	II. Late varieties								
	Tuscan	—	89·3	83·0	92·9	10·7		6·3	17·0
	Hunters	—	92·5	82·2	89·0	7·5		10·3	17·8
	Velvet	—	93·3	83·1	89·3	6·7		10·2	16·9
	Yeoman II	—	93·4	84·9	91·0	6·6		8·5	15·1
	Victor	—	95·2	85·3	89·6	4·8		9·9	14·7
1931–2	I. Early varieties								
	Tuscan	95·3	96·1	92·8	96·1	4·7	(+0·8)	3·3	7·2
	Solid-Straw Velvet	97·0	91·2	88·5	97·0	3·0	5·8	2·7	11·5
	Major	95·4	92·3	88·1	95·4	4·6	3·1	4·2	11·9
	Dreadnought	95·2	89·5	87·5	97·8	4·8	5·7	2·0	12·5
	S.E.	1·15	1·35	—	1·23	—	—	—	—
	II. Late varieties								
	Tuscan	96·8	97·0	90·9	93·7	4·0	(+1·0)	6·1	9·1
	Squarehead's Master	95·0	92·5	88·8	96·0	5·0	2·5	3·7	11·2
	Hunters	97·0	89·4	87·5	97·9	3·0	7·6	1·9	12·5
	Velvet	96·3	80·9	78·9	97·6	3·7	15·4	2·0	21·1
	Yeoman II	96·0	89·0	85·4	96·0	4·0	7·0	3·6	14·6
	Victor	93·9	81·3	77·6	95·5	6·1	12·6	3·7	22·4
	S.E.	1·02	1·25	—	2·04	—	—	—	—
	III. High-quality varieties								
	Tuscan	96·0	95·8	93·9	98·0	4·0	0·2	1·9	6·1
	Marquis	94·7	87·5	86·0	98·2	5·3	7·2	1·5	14·0
	Reward	93·8	90·8	86·2	95·0	6·2	3·0	4·6	13·8
	Garnet	94·0	86·8	83·2	95·8	6·0	7·2	3·6	16·8
	White Fife	96·5	90·2	86·5	95·9	3·5	6·3	3·7	13·5
	Tenmarq	92·4	82·2	79·5	96·7	7·6	10·2	2·7	20·5
	S.E.	0·93	1·68	—	1·70	—	—	—	—
1932–3	I. Early and late varieties								
	Tuscan	99·0	99·2	98·6	99·5	1·0	(+0·2)	0·6	1·4
	Cross 7	97·5	97·2	97·3	100·0	2·5	0·3	(+0·1)	2·7
	Solid-Straw Velvet	98·9	96·2	93·7	97·5	1·1	2·7	2·5	6·3
	Squarehead's Master	99·0	98·6	95·2	96·6	1·0	0·4	3·4	4·8
	Hunters	99·2	98·6	95·4	96·8	0·8	0·6	3·2	4·6
	Yeoman II	97·8	97·0	87·0	89·7	2·2	0·8	10·0	13·0
	Victor	98·4	97·3	91·4	94·0	1·6	1·1	5·9	8·6
	S.E.	0·63	—	—	—	—	—	—	—
	II. High-quality varieties								
	Tuscan	98·4	99·3	96·9	97·6	1·6	(+0·9)	2·4	3·1
	Reward	96·9	97·6	86·6	88·8	3·1	(+0·7)	11·0	13·4
	Garnet	98·0	98·4	96·4	98·0	2·0	(+0·4)	2·0	3·6
1933–4	I. Early and late varieties								
	Tuscan	94·3	95·4	95·4	100·0	5·7	(+1·1)	0·0	4·6
	Cross 7	94·3	94·8	93·5	97·9	5·7	(+0·5)	1·3	6·5
	Hunters	97·2	97·3	96·9	99·5	2·8	(+0·1)	0·4	3·1
	Yeoman II	96·8	97·1	96·8	99·6	3·2	(+0·3)	0·3	3·2
	S.E.	1·11	—	—	—	—	—	—	—
	II. High-quality varieties								
	Tuscan	96·6	96·7	96·7	100·0	3·4	(+0·1)	0·0	3·3
	Jumbuck	94·2	95·6	92·5	96·8	5·8	(+0·6)	3·1	7·5
	S.E.	0·87	—	—	—	—	—	—	—

varieties. Observation showed that in numerous plants of the affected varieties the rhizome was blackened and severed about ½–1 in. below the tillering node. There was no indication of insect damage[1]. This winter being exceptionally wet, with extended cold spells, it is thought that the phenomenon was caused by a combination of unfavourable environmental circumstances, and that the differential losses were due to relative varietal susceptibility to these conditions.

The losses between seedling count and harvest were small and evenly distributed.

In 1932–3 germination and seedling survival were high throughout, but Yeoman and Reward suffered heavily in the later period of growth. It is difficult to give a reason for this, unless it be the excessive rainfall in October.

In summarising, it can be stated that characteristic varietal differences in seedling and plant mortality have been found, and that in seasons with differential mortality Tuscan was the variety with the smallest losses.

(2) *Tiller development.*

Side tiller counts started as soon as any variety showed signs of tillering, and were conducted periodically until the end of tillering became evident[2].

Although counts were intended to take place in 10-day periods, the incidence of Sundays and of bad weather varied the lengths of intervals.

Table III contains the number of side tillers per plant. The asterisks between any two sets of figures indicate the period when the number of tillers produced reached the number of heads matured ("Critical period of tillering", see p. 478).

In the following, the chief elements of tillering will be discussed for each of the three groups of varieties. These elements are:

Start of tillering.
Tillering up to critical period.
Tillering after critical period.
Maximum number of tillers produced.
Tiller survival (percentage of tillers producing mature heads).

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[1] An attempt to strike pathogenic micro-organisms from affected plants, made by Mr J. W. Calder, did not produce any known foot-rot organisms.

[2] The end of the tillering season was taken to have occurred when two subsequent counts gave identical, or almost identical results. This was confirmed by subsequent observations which showed the absence of young tillers. In some experiments (chessboards 1931–2, see also census trials, Table XI, 1933–4) the last count returned considerably smaller figures than the previous one. This was due to the latest tillers concluding their development at a very early stage and being covered by rapidly growing leaves.

Maximum tiller production (number of side tillers + 1·00, for the main tiller) and tiller survival are listed in Table IV.

In the *early varieties*, both the inception of tillering and tiller development up to the critical period are closely similar in all varieties included. The critical period falls at the end of the first month after the beginning of tillering, with the exception of Major, and, in one season of Dreadnought, which reach the critical period earlier. After the critical period,

Table III. *Chessboard trials: number of side tillers per plant.*

Date	I. Early and high-quality varieties					II. Late varieties				
1930	T.	S.S.V.	Maj.	D.	M.	T.	H.	V.	Y.	Vic.
Aug. 1	0	0·03	0·04	0·01	0·06	0·01	0·01	0	0·39	0·01
„ 9	0·13	0·16	0·19	0·13	0·31	0·03	0·13	0·01	0·55	0·05
„ 18	0·81	1·01	0·93	0·65	0·88	0·28	0·79	0·34	1·44	0·43
			***	***			***		***	
„ 26	1·99	2·16	2·02	1·69	1·91	1·06	1·64	1·09	2·28	1·31
		***			***					***
Sept. 4	2·41	2·54	2·70	2·21	2·32	1·65	1·97	1·54	3·00	1·89
	***							***		
„ 15	3·39	3·36	3·45	2·66	2·66	2·02	2·54	2·08	4·03	2·31
						***				
„ 23	4·54	5·01	4·63	3·79	3·79	2·84	3·40	2·82	6·23	2·85
Oct. 2	6·04	6·85	6·97	5·62	6·10	4·36	6·48	5·42	9·88	4·55
„ 11	5·99	7·20	7·28	5·87	7·63	4·67	7·40	6·30	12·40	5·98
„ 21	6·07	7·20	7·63	5·85	7·95	4·60	7·15	6·33	12·65	6·17

Date	I. Early varieties				II. Late varieties					
1931	T.	S.S.V.	Maj.	D.	T.	S.M.	H.	V.	Y.	Vic.
July 22	0·15	0·20	0·55	0·22	0·22	1·20	0·87	0·37	1·37	0·40
„ 31	0·53	0·83	1·16	0·88	0·77	1·64	1·42	0·87	1·84	0·84
Aug. 10	1·28	1·48	1·75	1·49	1·50	2·17	1·83	1·37	2·29	1·29
			***			***	***		***	
„ 20	2·13	2·25	2·32	2·22	2·42	2·63	2·32	1·97	3·29	1·90
	***	***		***	***			***		***
Sept. 4	3·51	3·98	3·72	4·00	3·95	5·38	4·91	3·35	6·26	4·22
„ 11	4·35	5·18	4·84	5·99	4·62	7·04	6·15	4·48	6·05	5·53
„ 21	5·42	7·05	7·08	7·52	5·73	9·86	8·65	7·05	11·36	7·86
Oct. 5	5·62	7·62	7·94	8·63	5·82	11·89	10·02	9·26	14·70	9·64
„ 21	5·18	6·18	6·32	7·51	5·37	10·80	8·87	8·14	12·52	8·51

Date	III. High-quality varieties					
1931	T.	M.	R.	G.	W.F.	Tq.
July 22	0·07	0·07	0·07	0·07	0·41	0·21
„ 31	0·40	0·20	0·29	0·23	0·91	0·47
Aug. 10	0·93	0·44	0·47	0·61	1·35	0·83
„ 20	1·95	1·50	1·27	1·57	1·81	1·50
		***			***	
Sept. 4	3·21	3·08	2·97	3·12	4·64	2·23
	***		***	***		***
„ 11	4·25	3·99	3·72	4·33	6·85	2·84
„ 21	6·45	6·97	5·75	7·16	10·11	4·74
Oct. 5	6·98	11·27	8·27	11·56	14·83	7·87
„ 21	6·90	9·14	6·94	9·77	12·66	7·76

Table III (*continued*).

Date 1932	I. Early and late varieties							II. High-quality varieties		
	T.	Cr. 7	S.S.V.	S.M.	H.	Y.	Vic.	T.	R.	G.
Aug. 10	0	0	0	0	0	0·10	0	0	0	0
,, 18	0·01	0·09	0·03	0·52	0·13	0·43	0	0	0	0
				***		***				
,, 30	0·68	1·14	0·66	1·60	1·00	1·65	0·47	0·21	0·15	0·35
					***		***			
Sept. 8	1·89	2·43	1·95	2·60	1·97	2·58	1·43	1·43	1·47	1·44
	***	***	***					***	***	***
,, 20	3·18	3·95	3·49	3·86	3·16	4·09	2·83	2·64	2·33	2·69
,, 29	4·09	4·33	4·32	4·39	4·00	5·08	3·34	3·91	3·15	3·72
Oct. 8	4·39	4·50	4·62	4·88	4·61	6·32	3·70	4·35	3·57	4·43
,, 19	4·48	4·68	4·82	4·94	4·85	6·90	3·73	4·54	4·07	4·98

Date 1933	I. Early and late varieties				II. High-quality varieties	
	T.	Cr. 7	H.	Y.	T.	J.
July 26	0·29	0·28	0·72	1·29	0·22	0·01
				***		
Aug. 7	1·09	1·14	1·44	2·12	0·82	0·12
			***			
,, 17	1·91	1·84	2·11	2·64	1·75	0·72
	***	***			***	
,, 28	3·00	3·11	3·60	4·24	2·74	2·03
						***
Sept. 7	4·16	4·80	6·10	7·15	3·51	2·65
,, 18	5·07	6·02	7·99	9·59	4·10	3·63
,, 29	5·06	5·80	7·97	9·54	4·17	3·67

Table IV. *Total number of tillers per plant, and tiller survival.*

	1930–1		1931–2		1932–3		1933–4	
Variety	Max. tillering	Survival %	Max. tillering	Survival %	Max. tillering	Survival %	Max. tillering	Survival %
I. Early varieties								
Tuscan	7·07	47·8	6·62	59·5	5·48	64·4	6·07	63·6
Solid-Straw Velvet	8·20	41·0	8·62	42·3	5·82	55·1	—	—
Major	8·63	29·9	8·94	35·0	—	—	—	—
Dreadnought	6·85	37·2	9·63	37·9	—	—	—	—
Cross 7	—	—	—	—	5·69	67·3	7·02	52·0
II. Late varieties								
(Tuscan)	5·67	56·3	6·82	57·9	5·48	64·4	6·07	63·6
Hunters	8·40	31·2	11·02	29·2	5·85	45·1	8·99	35·6
Squarehead's Master	—	—	12·89	22·2	5·94	40·7	—	—
Velvet	7·30	38·3	10·26	32·7	—	—	—	—
Yeoman	13·40	19·5	15·70	21·3	7·90	37·3	10·54	25·7
Victor	6·98	33·6	10·64	30·1	4·73	45·2	—	—
III. High-quality varieties								
(Tuscan)	7·07	47·8	7·98	54·0	5·54	63·3	5·17	70·5
Marquis	8·95	36·4	12·27	32·0	—	—	—	—
Reward	—	—	9·27	42·2	5·07	60·6	—	—
Garnet	—	—	12·56	37·3	5·98	59·8	—	—
White Fife	—	—	15·83	21·6	—	—	—	—
Tenmarq	—	—	8·87	42·9	—	—	—	—
Jumbuck	—	—	—	—	—	—	4·67	75·2

however, and up to the end of the tillering process, some characteristic varietal differences are revealed. Whilst all varieties concerned belong to the sparsely tillering, erect or semi-erect type, as a rule the tiller production is lower in Tuscan than in the other varieties, with the exception of Dreadnought in one season only. Since, on the other hand, Tuscan generally produces the largest number of heads per plant, the combination of both these factors—low tiller production and high ear production—finds expression in its high rate of "tiller survival" (Table IV). With the exception of Cross 7 (in one season) Tuscan leads all the early, and incidentally also all the late varieties in every season. The lowest tiller survival is recorded for Major, with the other varieties intermediate.

All the *late varieties* included are semi-prostrate to prostrate in tillering habit. Their inception of tillering is earlier than in Tuscan, and accordingly most of them reach the critical period at an earlier date. The total number of tillers, with the exception of Victor in one season, is considerably higher, and, owing to this and to their lower rate of head production, their tiller survival is in comparison with Tuscan lower throughout.

Intervarietal comparison amongst the varieties of this group is summarised below. With the exception of the time of incidence of the critical period, there is little seasonal variation affecting the relative positions of varieties:

Beginning of tillering	Yeoman > Squarehead's Master > Hunters > Victor = Velvet.
Incidence of critical period	Yeoman > Squarehead's Master = Hunters > Victor > Velvet.
Total number of tillers produced	Yeoman > Squarehead's Master > Hunters > Victor = Velvet.
Tiller survival	Yeoman < Squarehead's Master < Hunters < Victor < Velvet.

The interrelations between the various phases of the tillering process are evident. They will be further discussed in connection with the analysis of yield characters.

In the inception of tillering, and in the incidence of the critical period, the *high-quality varieties* resemble Tuscan, but there is a tendency to later development amongst some of them. When conditions are favourable for tiller development, as in 1930 and in 1931, those varieties produce a larger number of tillers compared with Tuscan. White Fife, the only prostrate variety in this group, is particularly prolific. Tiller survival is lower than in Tuscan in the two seasons mentioned above, but practically equal otherwise. Jumbuck differs remarkably from the rest in its late and sparse tillering and high rate of tiller survival.

Seasonal variation in the amount of tillering is pronounced, but the number of experimental seasons is hardly sufficient to permit of generalisations. The most striking difference is that occurring between 1932 and 1933, a season of remarkably low tillering values, and the rest, particularly 1931–2. The obvious explanation is the late sowing date in the former (June 2) and the early one in the latter (May 5). It will be seen that the late, high-tillering, varieties were more affected by seasonal variation than the early, low-tillering forms. The following varieties were included in all four seasons (Table IV A). Whilst Hunters varies from 53·0 and 64·8 to 100 per cent., and Yeoman from 50·3 and 52·3 to 100 per cent., Tuscan merely varies from 74·3 and 77·5 to 100 per cent. The means also illustrate the relative amount of seasonal variation.

Table IV A. *Number of tillers, and tiller survival, for four seasons, expressed in percentages of the highest values* (% *in italics*).

	1930–1		1931–2		1932–3		1933–4		Mean	
Variety	Max. tillering	Survival %	Max. tillering	Survival %	Max. tillering	Survival %	Max. tillering	Survival %	Max. tillering	Survival %
Tuscan	7·07	47·8	6·62	59·5	5·48	64·4	6·07	63·6	—	—
	100	*74·3*	*95·6*	*92·5*	*77·5*	*100*	*85·9*	*98·9*	*89·7*	*91·4*
Hunters	8·40	31·2	11·02	29·2	5·85	45·1	8·99	35·6	—	—
	76·1	*69·2*	*100*	*64·8*	*53·0*	*100*	*81·5*	*79·0*	*77·6*	*78·2*
Yeoman	13·40	19·5	15·70	21·3	7·90	37·3	10·54	25·7	—	—
	85·5	*52·3*	*100*	*57·1*	*50·3*	*100*	*67·2*	*69·0*	*75·7*	*69·6*

Tiller production and tiller survival show a negative correlation. In seasons of low tillering the survival rate is high, and *vice versa*, so that the existence of a regulatory mechanism becomes evident which keeps the rate of ear production down to a certain characteristic level.

A case of remarkable local variation appears in the data for Tuscan in the two chessboards 1930–1. They were sown side by side on May 15 and May 19 respectively. The critical period was about three weeks later in the standard of chessboard II and the number of side tillers only 76 per cent. of that in chessboard I.

(3) *The mature population.*

The unit of comparative yielding capacity in these hand-sown trials is the average yield per plant (Table V). It must be considered whether plant yields in hand-sown experiments, with a spacing usually high above that of drilled field crops, can be used as indices of the yielding capacity of varieties under field conditions. This has been affirmed by Raum (1930) and rejected by Boekholt (1931), Heuser (1932), and others, the views

Table V. *Chessboard trials: yield attributes of mature population.*

(*a*) *Early varieties.*

	1929–30			1930–1					1931–2					1932–3				1933–4		
Yield attributes	T.	S.S.V.	S.E.	T.	S.S.V.	Maj.	D.	S.E.	T.	S S.V.	Maj.	D.	S.E.	T.	S.S.V.	Cr. 7	S.E.	T.	Cr. 7	S.E.
Average yield per plant (gm.)	2·71	3·06	0·132	3·42	4·01	4·27*	3·23*	0·116	3·71	3·49	†	4·24	0·085	3·70	3·48	3·79	0·108	4·77	4·13	0·059
Average yield per ear (gm.)	1·05	1·41	0·031	0·99	1·24	1·55*	1·24*	0·022	0·93	0·98	†	1·13	0·018	1·11	1·10	1·04	0·044	1·25	1·14	0·022
Average number of ears per plant	2·58	2·17	0·090	3·46	3·24	2·75	2·62	0·068	3·97	3·56	3·11	3·76	0·056	3·35	3·16	3·64	0·061	3·82	3·62	0·059
Average number of grains per plant	53·91	68·93	—	76·40	106·40	87·53*	61·18*	—	85·08	103·13	†	87·80	—	78·76	95·08	89·22	—	95·61	89·81	—
Average number of grains per ear	21·11	31·74	0·545	22·08	32·84	31·83*	23·35*	0·469	21·43	28·97	†	21·34	0·316	23·51	30·09	24·51	0·650	25·03	24·81	0·431
Average weight of 1000 grains (gm.)	49·71	44·36	0·367	44·90	37·72	48·77	53·00	0·372	43·51	33·84	46·94	52·79	0·384	47·35	36·80	42·72	0·705	50·00	45·90	0·448

(*b*) *Late varieties.*

	1929–30				1930–1						1931–2						
Yield attributes	T.	H.	Y.	S.E.	T.	H.	V.	Y.	Vic.	S.E.	T.	H.	S.M.	V.	Y.	Vic.	S.E.
Average yield per plant (gm.)	2·71	2·92	2·39	0·132	3·43	†	†	3·02*	3·55*	0·114	3·97	3·45	2·98	3·44	3·19	3·71	0·103
Average yield per ear (gm.)	1·05	1·40	1·28	0·031	1·02	†	†	1·08*	1·46*	0·028	0·96	1·03	1·02	1·04	0·93	1·15	0·026
Average number of ears per plant	2·58	2·09	1·87	0·090	3·36	2·60	2·80	2·79	2·43	0·084	4·12	3·36	2·92	3·32	3·42	3·22	0·064
Average number of grains per plant	53·91	58·22	48·23	—	74·56	†	†	71·90*	85·80*	—	87·96	77·82	63·16	86·15	75·38	90·61	—
Average number of grains per ear	21·11	27·87	25·70	0·545	22·22	†	†	25·77*	35·31*	0·579	21·35	23·16	21·63	25·95	22·04	28·14	0·482
Average weight of 1000 grains (gm.)	49·71	50·22	49·53	0·367	46·32	†	†	41·80	41·67	0·388	45·23	44·48	46·96	40·22	42·25	40·99	0·505

Table V (*continued*).

Yield attributes	1932–3						1933–4			
	T.	H.	S.M.	Y.	Vic.	S.E.	T.	H.	Y.	S.E.
Average yield per plant (gm.)	3·70	3·12	2·65	2·69	2·86	0·108	4·77	3·97	3·35	0·059
Average yield per ear (gm.)	1·11	1·26	1·22	1·03	1·31	0·044	1·25	1·29	1·20	0 022
Average number of ears per plant	3·35	2·47	2·17	2 61	2·19	0·061	3 82	3·06	2·79	0·059
Average number of grains per plant	78·76	74·50	62 37	69·37	77·91	—	95·61	85·04	72·07	—
Average number of grains per ear	23·51	30·16	28·74	26·58	35·66	0·650	25·03	27·79	25 83	0·431
Average weight of 1000 grains (gm.)	47·35	41·99	42·69	38·70	36·76	0·705	50·00	46·55	46 50	0·448

(*c*) *High-quality varieties.*

Yield attributes	1929–30			1930–1			1931–2							1932–3				1933–4		
	T.	R.F.	S.E.	T.	M.	S.E.	T.	M.	R.	G.	W.F.	Tq.	S E.	T.	R	G.	S.E.	T.	J.	S.E.
Average yield per plant (gm.)	2·71	2·38	0·132	3·42	2·79	0·116	3·83	2·90	‡	‡	2 56	3·35	0·081	3·53	2·61	2·84	0·140	4·58	4·17	0·175
Average yield per ear (gm.)	1·05	0·99	0·031	0·99	0·87	0·022	0·90	0·72	‡	‡	0·73	0 91	0 012	1·01	0·84	0·80	0 033	1·24	1·20	0·045
Average number of ears per plant	2·58	2·30	0·090	3 46	3·21	0·068	4·25	4·03	4·18	4·56	3·51	3·69	0 069	3·49	3·09	3·56	0·100	3·71	3·49	0·054
Average number of grains per plant	53·91	60·93	—	76·40	81·41	—	86 45	80·92	‡	‡	72·48	81·07	—	75·53	72·99	89·52	—	96·43	82·66	—
Average number of grains per ear	21·11	26·46	0·545	22·08	25·36	0·469	20·34	20·08	‡	‡	20·65	21·97	0·248	21·07	23·62	25 09	0·360	24·54	22·96	0·834
Average weight of 1000 grains (gm.)	49·71	37·52	0·367	44·90	34·19	0·372	44·31	35·62	27·38	23·45	35·36	41·27	0·325	47·95	35·63	31·96	0·884	50·37	52·01	0·883

* Grain slightly shaken. † Grain badly shaken. ‡ Grain damaged by birds.

of the opposing investigators being determined by their appreciation of tillering and head formation as regulatory or as varietal characteristics.

It requires further consideration whether the components of plant yield are likely to show similar performance under field and plot conditions. Both these questions have been put to the test by Engledow and Ramiah (1930), who used hand-sown plots with even spacing with two rates of seeding, in comparison with census samples from drilled crops grown under similar conditions.

In both sets of tests the varieties included showed similar differences in plant yields and yield components; but in the hand-sown trials all values are higher, chiefly due to better cultivation. The same method was applied in the trials reported below, with similar results. Provided the varietal differences are comparatively wide, hand-sown trials with

Table VI. *Yields in drilled varieties at Lincoln laid down on the same field as the analytical yield trials (statistically significant differences are italicised).*

Season	Variety	Yield	Difference from Tuscan*	Difference from Tuscan*, in % of Tuscan yield	
		(bushels per acre)		Drilled trials	Chess-boards
1929–30	S.S.V.	30·9†	+ *5·5*	+ *21·6*	+ *12·8*
	H.	48·9	+ *2·9*	+ *6·3*	+ 7·7
	Y.	43·7	− *3·6*	− *7·6*	− *11·7*
	R.F.	35·5	− *4·9*	− *12·1*	− *12·0*
1930–1	S.S.V.	45·5	+ *4·8*	+ *11·8*	+ *17·1*
	Maj.	37·4‡	− 1·8	− 4·6	+ *25·0*
	D.	29·3§	− *14·8*	− *33·6*	− 5·5
	H.	42·9‡	− *5·7*	− *11·7*	—
	V.	30·9§	− *7·4*	− *19·3*	—
	Y.	41·2	− *8·2*	− *16·6*	− *12·0*
	Vic.	49·9	+ 2·1	+ 4·4	+ 3·4
	M.	30·0	− *7·4*	− *19·8*	− *18·3*
1931–2	S.S.V.	63·7	+ 1·7	+ 2·7	− *5·8*
	D.	70·4‡	+ *4·4*	+ *6·7*	+ *14·1*
	H.	49·2§	− *17·5*	− *26·2*	− *13·0*
	V.	48·2	− *11·6*	− *19·4*	− *13·3*
	Y.	50·7	− *17·0*	− *25·1*	− *19·5*
	Vic.	53·4	− *13·6*	− *20·3*	− *6·5*
1932–3	S.S.V.	73·8	− *9·0*	− *10·9*	− *5·8*
	Cr. 7	88·5	− 0·5	− 0·6	+ 2·4
	H.	66·3	− *16·2*	− *19·7*	− *15·5*
	Y.	61·8	− *23·9*	− *27·9*	− *27·2*
1933–4	Cr. 7	43·4	− 1·0	− 2·3	− *13·3*
	H.	45·2	+ 0·3	+ 0·7	− *16·7*
	J.	34·7	− *10·2*	− *22·8*	− *9·0*

* "Differences from Tuscan" are derived from the difference between the mean of the ten plot yields of the variety and that of the adjacent Tuscan plots (Hudson's modification of Beaven's half-drill-strip method).

† On low-lying land, water-logged during the winter.

‡ Some grain shed.

§ Much grain shed.

rates of seeding not disproportionately below that of field crops appear to reflect the characteristic differences exhibited in the latter; but these differences are as viewed through a magnifying glass producing an image not always free from distortion.

A subsidiary check on the validity of plant yields for comparisons was obtained from the yields of drilled field trials laid out every year on the same field, under conditions identical with those of the hand-sown trials.

In comparing the relative yields in field trials recorded in Table VI with the yields per plant in Table V—see also Table VI, columns 5 and 6—it will be found that in general there obtains a close correspondence between the yield relations observed in the drilled and in the hand-sown trials. The yielding capacity of varieties in comparison with Tuscan and amongst themselves is as a rule fairly similar in both sets. In three instances, however, the differences are considerable. Major and Dreadnought in 1930-1 yielded in the chessboard considerably higher, in comparison with Tuscan, than under field conditions, which can be satisfactorily explained by the large losses due to shedding suffered by the field crop, whereas the small hand-sown plots could be more efficiently safeguarded. Further, the hand-sown Hunters crop in 1933-4 yielded less than Tuscan, whereas the yields in the field trial were equal. But otherwise the performances in both sets of trials are parallel to a high degree.

(*a*) *Early varieties* (Table V (*a*)).

The most interesting comparison in this group is that between Tuscan and Solid-Straw Velvet. This variety is reputed to return higher yields than Tuscan where conditions are favourable. It is fairly resistant to shedding of grain, though not to the same extent as Tuscan. Its chief disadvantage is the lower baking value of its grain. Still, in crosses with high-quality forms this may not be sufficient to offset the advantages of valuable yield factors entering into the cross. In two seasons out of four Tuscan was superior in yield, in the other two Solid-Straw Velvet. In all four Solid-Straw Velvet produced a smaller number of heads, the proportion varying between narrow limits (84–94 per cent. of the ear formation of Tuscan). The yield differences are almost entirely due to the difference in the average yield per ear. Whereas that of Tuscan remains comparatively constant from year to year, varying between 0·93 and 1·11, that of Solid-Straw Velvet varies between 0·98 and 1·41 gm. Obviously Solid-Straw Velvet is more affected by environmental conditions than is Tuscan. A further analysis shows that these differences are about equally due to the relative development of grain number and grain size.

Solid-Straw Velvet in percentage of Tuscan.

	Average 1929–30 and 1930–1 (S.S.V. superior yield)	Average 1931–2 and 1932–3 (S.S.V. inferior yield)
Number of grains per ear	149·5	131·4
Weight of 1000 grains gm.	86·7	77·7

The seasonal variations of Solid-Straw Velvet with respect to grain number, about the same as that of Tuscan, is not nearly as strong as that in grain size.

Lowest seasonal value in percentage of highest.

	Number of grains per ear	Weight of 1000 grains
Tuscan	89·7	87·5 gm.
Solid-Straw Velvet	88·3	69·3

It can be concluded from this analysis that Solid-Straw Velvet is inferior to Tuscan in the number of heads produced; that it has to rely on the formation of a large number of grains per ear, and on a good development of its grain. Under conditions which do not encourage a full development of these characteristics, its yield will fall markedly below that of Tuscan. The latter exhibits a more constant development of the number, and particularly of the size of grains; it is to a lesser extent influenced by environmental conditions. It seems impossible to associate the seasonal variation with any individual environmental factor, climatic or otherwise.

Cross 7 in most respects closely resembles Tuscan. An analysis of the data has been given elsewhere (Frankel, 1934). Major and Dreadnought resemble the "late varieties" in the make-up of the mature plant, though belonging to the "early varieties" in view of their times of ripening and their tillering modes. The data for both varieties, scanty as they are, demonstrate the formation of markedly fewer heads than Tuscan, but superiority in the yield per head, generally resulting in a higher yield per plant. This is, in farming practice, largely offset by excessive shattering of grain, and low baking value. Still, on some classes of the best of land, where the full advantages of large ear development can regularly be reaped, Dreadnought is the most advantageous among New Zealand wheats.

It is surprising to see on further analysis of the yield per ear, that

Dreadnought is not generally superior to Tuscan in the number of grains per head, but owes its high yield chiefly to a very large grain. It is to be expected that in its own habitat, *i.e.* in South Canterbury, where conditions of soil and rainfall are more favourable, the number of grains per head is larger. Major, on the other hand, excels Tuscan in both characteristics, though in grain size not to the same extent as Dreadnought.

It can be concluded that Dreadnought and Major rely for the achievement of high yields on the development of large heads to an even greater extent than Solid-Straw Velvet, and that they are not likely to be successful where conditions do not favour the formation of a large head, and in particular of a large grain.

(*b*) *Late varieties* (Table V (*b*)).

With the exception of two instances—Hunters in 1929–30 and Victor in 1930–1—Tuscan exceeds in yield per plant all the late varieties in every season. This is in general agreement with the results of drilled trials on the same field (see above, Table VI). It is due to the smaller number of heads produced by the "late varieties" which only rarely is balanced by a proportionately heavier head. With few exceptions—chiefly the two cases mentioned above—the yield per ear is only slightly higher than that of Tuscan, and in one instance, viz. Yeoman in 1931–2, it is even lower. The number of grains per ear generally is larger than that of Tuscan, the grain size varies a good deal, in the majority of cases considerably more than in Tuscan. Since these varieties frequently produce less grains per plant than Tuscan, their grain would have to be as large or larger than Tuscan's to equal it in yield, but this condition is only rarely fulfilled by any of the varieties, viz. Hunters in two seasons, and Yeoman and Squarehead's Master each in one season.

Among themselves, the late varieties exhibit few consistently recurring differences. Victor has a larger number of grains per ear than any of the other varieties, as is to be expected from the shape of its head. Hunters and Yeoman generally form more heads than Victor and Squarehead's Master. In plant yield, Hunters is superior to Yeoman and Squarehead's Master, to the former owing to a stronger development with respect to all yield components, to the latter chiefly owing to its larger number of heads. This comparatively high rate of head formation is perhaps at least partially responsible for its success in New Zealand (see below).

The two New Zealand varieties of this group, Hunters and Velvet, are, so far as the limited data will permit one to judge, closely similar in most respects. There is an indication of Velvet having a larger number of grains of a smaller size in comparison with Hunters.

(*c*) *High-quality varieties* (Table V (*c*)).

Plant yields are low for all members of this group as is to be expected from variety trials and general farming experience. Among the Canadian varieties (with the exception of White Fife) head formation is close to that of Tuscan, with Garnet in the lead and Marquis, Reward and Red Fife following. White Fife, included in one season only, ranges well below this group, about equal with Tenmarq.

The Canadian varieties and Tenmarq are equal—and more frequently superior—to Tuscan in the number of grains per ear, in several instances resulting in a larger grain number per plant than obtains for Tuscan. But the grain is so small—with Garnet the smallest and Tenmarq the largest—that the resulting plant yields are low. It is then important to note that these varieties produce a satisfactory number of heads and of grains—sometimes even excelling Tuscan in both respects (Garnet)—but that their grain size is too small to facilitate satisfactory yields.

Jumbuck, the Australian variety included, yields less than Tuscan, in spite of its grain size which is even larger than that of Tuscan. Its chief defect is the smaller number of heads. Whether a higher rate of seeding in comparison with Tuscan would make up for it is doubtful in view of the number of grains per head which, contrary to expectation, is smaller than that of Tuscan even under the conditions of liberal spacing provided in this trial. Its large size of grain must be considered as a valuable asset for combination work.

B. SPACING TRIALS.

In 1929–30 the varieties included were Tuscan, Hunters and Yeoman, to which Solid-Straw Velvet was added in 1930–1. Spacing, rate of seeding and number of plants were as follows:

Spacing in.	Corresponding rate of seeding per acre lb.	Number of plants per row (15 ft.)	Number of rows per plot (not counting guard rows)
½	177	360	2
1¼	73	140	2
2	44	90	2
4	22	45	3
12	7·5	15	4

Number of replications, sowing dates, methods of sowing and harvesting, etc., were given on pp. 472, 473.

(1) *Germination, plant survival and tiller development.*

Developmental determinations are restricted to one season only (1930–1). Field germination (Table VII) was similar in all varieties and in all spacings, with the exception of the 12-in. spacing, for which germination is higher in all varieties. The differences are small and are doubtless due to the fact that in order to insure a complete stand in the widely spaced 12-in. plots, two seeds were sown side by side, and one removed after germination.

Plant survival was determined for the three intermediate spacings only. It does not clearly reflect an influence of spacing in any of the four varieties, although there seems to be an indication in Tuscan of increasing survival with wider spacing. The question whether plants can be "crowded out", *i.e.* killed by excessive density, could not be approached in this trial, since the ½-in. spacing did not facilitate a reliable plant count. But in the 1¼-in. spacing—which must frequently occur under field conditions—the plant losses under the conditions of this experiment were no greater than in the wider spacings.

During the earlier part of the tillering period, viz. till about September 20, the tillers of Tuscan, Hunters and Solid-Straw Velvet develop at a similar pace, though Solid-Straw Velvet is first off the mark on August 9 (Table VIII). From then onward, the differences become marked in the intermediate spacings, the ascending order being Tuscan, Solid-Straw Velvet, Hunters. In the ½-in. and 12-in. spacing, however, Tuscan and Solid-Straw Velvet are almost equal, and Hunters is only slightly superior, which indicates that, in comparison with the other two varieties, Tuscan is most vigorous under extreme conditions of spacing. Yeoman is markedly superior throughout the whole period in all spacings, this superiority decreasing with growing space. Its maximum tiller-producing capacity is apparently not vastly higher than that of the other varieties as one would be led to believe from observations on field and chessboard trials.

Tiller survival presents itself as a strong varietal characteristic throughout the range of spacings (Fig. 1), the order being Tuscan, Solid-Straw Velvet, Hunters, Yeoman. But it is the relation between tillering induced by increased space, and the corresponding greater or smaller increase in head formation, which opens up a better understanding of varietal differences.

Tuscan shows a rapid increase of tiller survival with an increase in space from ½ to 1¼ in. (Fig. 1). At 2 in. almost the same level is main-

Table VII. *Field germination and rate of plant survival in the spacing trial*, 1930–1.

Spacing in.	Field germination (%)					Plant survival									
						% of grain sown					% of plants germinated				
	T.	H.	S.S.V.	Y.	Mean	T.	H.	S.S.V.	Y.	Mean	T.	H.	S.S.V.	Y.	Mean
½	82·1	82·5	82·1	82·1	82·2	—	—	—	—	—	—	—	—	—	—
1¼	84·8	83·3	84·1	80·4	83·2	74·2	80·6	80·0	—	78·3	91·6	96·7	95·2	—	94·2
2	79·6	79·2	82·9	82·9	81·2	76·4	77·8	78·2	79·6	78·0	95·9	98·2	94·4	96·1	96·2
4	84·3	86·1	81·5	82·4	83·6	82·7	80·9	76·5	82·7	80·4	98·2	93·9	93·9	100·0	96·5
12	86·6	88·0	87·5	88·4	87·6	—	—	—	—	—	—	—	—	—	—
Mean	83·5	83·8	83·6	83·2	83·9	77·8	79·8	78·2	81·1	78·9	95·3	96·3	94·5	98·1	95·6

Table VIII. *Number of side tillers and rate of tiller survival in the spacing trial*, 1930–1.

(Stars indicate the critical period, phase (*a*), lines the phase (*b*); see p. 499.)

Date	Number of side tillers per plant																			
	½ in.				1¼ in.				2 in.				4 in.				12 in.			
	T.	H.	S.S.V.	Y.	T.	H.	S.S.V.	Y.	T.	H.	S.S.V.	Y.	T.	H.	S.S.V.	Y.	T.	H.	S.S.V.	Y.
Aug. 9	0·05	*** 0·09	*** 0·18	*** 0·84	0·10	0·09	0·19	0·85 ***	0·10	0·10	0·25	0·68	0·09	0·05	0·25	0·89	0·09	0·10	0·28	0·76
„ 20	0·24 ***	0·50	0·38	1·70	0·34	0·575 ***	0·84 ***	1·81	0·59	0·83 ***	0·70	1·61 ***	0·50	0·84	0·73	1·59	0·66	0·89	0·85	1·89
Sept. 1–2	1·23	1·64	0·96	2·91	1·43 ***	1·73	1·71	3·59	1·75	1·94	1·66 ***	2·78	1·98	2·05	2·09	3·35 ***	2·10	1·95	1·84	3·03
„ 15	2·05	2·09	1·84	3·63	2·06 ——	2·31 ——	2·30 ——	4·41 ——	2·45 ***	2·52	2·33	4·36	2·48	2·65 ***	2·60 ***	4·49	2·91	2·99	2·55	4·26
„ 24	2·59	2·96	2·70	4·83	3·36	4·16	3·68	8·10	4·14 ——	4·29 ——	4·09 ——	6·90	4·54 ***	4·83	4·44	8·28	4·60	5·48	4·30	7·31 ***
Oct. 2	2·75	2·89	2·69	—	3·86	5·85	5·26	11·71	5·94	7·10	6·19	10·33	6·48 ——	7·35 ——	6·78 ——	12·24 ——	7·49 ——	8·75 ***	6·70 ***	11·00
„ 13	2·52	2·74	2·33	—	3·74	6·04	5·38	12·36	5·75	8·38	7·70	13·45	8·41	11·13	10·23	18·35	12·96 ***	14·70	12·90	18·11 ——
„ 23	—	—	—	—	—	—	—	—	6·06	8·48	7·83	14·06	8·83	10·91	11·49	18·66	16·15	16·99	15·30	20·79
„ 30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17·73	18·24	16·39	22·28
Max. number of tillers	3·75	3·89	3·69	5·83	4·74	7·04	6·38	13·36	7·06	9·48	8·83	15·06	9·83	11·91	12·49	19·66	18·73	19·24	17·39	23·29
Heads per plant (harvest)	1·26	1·01	1·06	1·01	2·52	1·63	2·07	2·01	3·47	2·67	3·20	2·93	5·72	4·67	5·24	4·88	14·09	11·51	11·50	9·60
% tiller survival	33·6	26·0	28·8	19·9	53·2	23·2	32·4	15·0	49·1	28·2	36·2	19·5	58·2	39·2	42·0	24·2	75·2	59·9	66·2	41·2

tained, with an even rise to 4 and 12 in. With an increase in space from ½ to 1¼ in., a Tuscan plant is induced to produce not merely the proportionately increased number of heads, but about 20 per cent. more. This distinguishes it clearly from the three other varieties. The graphs for Hunters and Yeoman run almost parallel. Instead of an increase in the survival of additional tillers, their 1¼-in. spacing even shows a slight decrease with a gradual rise at 2 and 4 in. and a steep increase at 12 in.

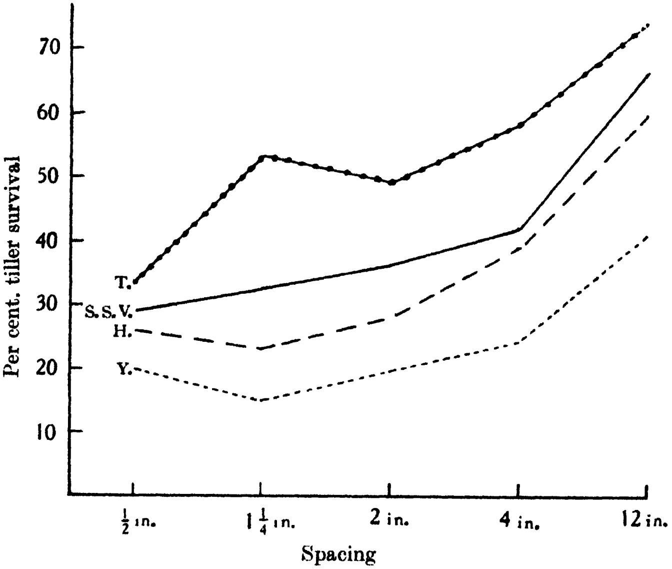

Fig. 1. Tiller survival of four varieties in the spacing trial, 1930–1.

Obviously the more profuse tillering under more favourable conditions does not—within the limits of the common field spacings—lead to a more than proportionate increase in head formation. Solid-Straw Velvet is intermediate between these extremes.

(2) *The mature population.*

Yield characteristics, per plot and per plant, for the first season have been recorded previously (Frankel, 1931), those for the second are found in Table IX. In Table X the records of both seasons are expressed in proportion of the values for the ½-in. spacing. The yield per one row is derived by multiplying the yield per plant with the theoretical number of plants.

Tuscan and Solid-Straw Velvet show even *plot yields* in all spacings between ½ and 12 in. (Table X). This demonstrates a high degree of adaptability. Hunters, on the other hand, cannot be relied upon in the extreme spacings: in 1929–30 the 12-in., in the following year the ½-in. spacing is well below the level of the intermediate spacings. The 12-in. spacing of Yeoman is low in both seasons and the ½-in. spacing in the first.

Table IX. *Spacing trial*, 1930–1*: mature population.*

	Corrected yields per 1 row (15 ft.) (1)			
in.	T.	H.	S.S.V.	Y.
½	327·6	223·2	320·4	309·6
1¼	309·6	273·6	342·7	275·0
2	329·4	272·7	353·7	325·8
4	286·7	277·2	345·6	299·7
12	321·9	283·8	317·7	225·5

	Yield per plant (2)					
in.	T.	H.	S.S.V.	Y.	ϵ_d	$2\epsilon_d$
½	0·91	0·62	0·89	0·86	0·032	0·06
1¼	2·15	1·90	2·38	2·21	0·121	0·24
2	3·66	3·03	3·93	3·62	0·147	0·29
4	6·37	6·16	7·68	6·66	0·367	0·73
12	21·46	18·92	21·18	15·03	0·719	1·43

	Number of ears per plant (3)					
in.	T.	H.	S.S.V.	Y.	ϵ_d	$2\epsilon_d$
½	1·27	1·04	1·07	1·02	0·026	0·05
1¼	2·46	1·79	2·14	1·91	0·116	0·23
2	3·55	2·76	3·23	3·02	0·050	0·10
4	5·38	4·33	5·03	4·72	0·104	0·21
12	12·93	10·84	10·96	9·33	0·330	0·66

	Yield per ear (4)					
in.	T.	H.	S.S.V.	Y.	ϵ_d	$2\epsilon_d$
½	0·72	0·59	0·84	0·84	0·023	0·05
1¼	0·88	1·06	1·11	1·16	0·027	0·05
2	1·03	1·10	1·22	1·20	0·045	0·09
4	1·18	1·42	1·53	1·41	0·061	0·12
12	1·66	1·75	1·93	1·61	0·045	0·09

	Number of grains per plant (5)			
in.	T.	H.	S.S.V.	Y.
½	20·8026	16·57	24·93	21·43
1¼	47·82	44·70	62·40	53·63
2	80·02	73·69	104·46	88·52
4	139·56	145·57	200·14	158·31
12	461·21	429·05	486·51	343·99

	Number of grains per ear (6)					
in.	T.	H.	S.S.V.	Y.	ϵ_d	$2\epsilon_d$
½	16·38	15·93	23·30	21·01	0·62	1·24
1¼	19·44	24·97	29·16	28·08	0·51	1·02
2	22·54	26·70	32·34	29·31	0·94	1·88
4	25·94	33·62	39·79	33·54	1·16	2·32
12	35·67	39·58	44·39	36·83	1·04	2·08

	Weight of 1000 grains (7)					
in.	T.	H.	S.S.V.	Y.	ϵ_d	$2\epsilon_d$
½	45·37	38·53	37·26	41·61	0·50	0·99
1¼	47·11	43·08	39·40	42·54	0·37	0·73
2	48·33	43·05	40·02	42·62	0·36	0·72
4	48·09	44·34	39·76	41·81	0·43	0·86
12	48·54	45·76	43·81	44·91	0·38	0·76

The gradual rise in *plant yields* with increased space does not exhibit consistent varietal differences. But a study of the elements of plant yields shows the different structural mechanisms producing these increased yields. In the first season Tuscan owes its increasing plant yields in the wider spacings more to enhanced head formation, Hunters and Velvet to head weights. In the second season, however, this difference

Table X. *Spacing trials,* 1929–30 *and* 1930–1. *Yield characteristics expressed in proportion of the ½-in. spacing.* 1929–30 *in roman print,* 1930–1 *in italics.*

	Corrected yield per 1 row (15 ft.) (1)				Yield per plant (2)			
in.	T.	H.	S.S.V.	Y.	T.	H.	S.S.V.	Y.
½	1·00	1·00	—	1·00	1·00	1·00	—	1·00
	1·00	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*
1¼	0·93	0·99	—	1·13	2·32	2·47	—	2·81
	0·95	*1·23*	*1·07*	*0·89*	*2·36*	*3·06*	*2·67*	*2·57*
2	0·93	0·94	—	1·08	3·70	3·74	—	4·32
	1·01	*1·22*	*1·10*	*1·05*	*4·02*	*4·89*	*4·42*	*4·21*
4	1·06	0·97	—	1·14	8·50	7·73	—	9·15
	0·88	*1·24*	*1·08*	*0·97*	*7·00*	*9·94*	*8·63*	*7·74*
12	0·94	0·81	—	0·83	22·51	19·48	—	20·01
	0·98	*1·27*	*0·99*	*0·73*	*23·58*	*30·52*	*23·80*	*17·48*

	Yield per ear (3)				Number of ears per plant (4)			
in.	T.	H.	S.S.V.	Y.	T.	H.	S.S.V.	Y.
½	1·00	1·00	—	1·00	1·00	1·00	—	1·00
	1·00	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*
1¼	1·39	1·54	—	2·00	1·67	1·60	—	1·41
	1·22	*1·80*	*1·32*	*1·38*	*1·94*	*1·72*	*2·00*	*1·87*
2	1·37	1·58	—	2·09	2·69	2·37	—	2·06
	1·43	*1·86*	*1·45*	*1·43*	*2·80*	*2·65*	*3·02*	*2·96*
4	1·89	1·96	—	2·32	4·48	3·92	—	3·94
	1·64	*2·40*	*1·82*	*1·68*	*4·24*	*4·16*	*4·70*	*4·63*
12	2·18	2·26	—	2·90	10·25	8·60	—	6·90
	2·30	*2·97*	*2·30*	*1·92*	*10·18*	*10·42*	*10·24*	*9·15*

	Number of grains per plant (5)				Number of grains per ear (6)			
in.	T.	H.	S.S.V.	Y.	T.	H.	S.S.V.	Y.
½	1·00	1·00	—	1·00	1·00	1·00	—	1·00
	1·00	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*	*1·00*
1¼	2·22	2·22	—	2·47	1·33	1·39	—	1·75
	2·30	*2·70*	*2·50*	*2·50*	*1·19*	*1·57*	*1·25*	*1·34*
2	3·56	3·35	—	3·81	1·32	1·41	—	1·85
	3·85	*4·45*	*4·19*	*4·13*	*1·38*	*1·68*	*1·39*	*1·40*
4	7·73	6·43	—	7·96	1·72	1·64	—	2·01
	6·71	*8·79*	*8·03*	*7·39*	*1·58*	*2·10*	*1·71*	*1·60*
12	20·45	15·91	—	17·09	1·99	1·85	—	2·47
	22·17	*25·89*	*19·52*	*16·05*	*2·18*	*2·48*	*1·91*	*1·75*

	Weight of 1000 grains (7)			
in.	T.	H.	S.S.V.	Y.
½	1·00	1·00	—	1·00
	1·00	*1·00*	*1·00*	*1·00*
1¼	1·04	1·12	—	1·13
	1·04	*1·12*	*1·06*	*1·02*
2	1·03	1·12	—	1·12
	1·07	*1·12*	*1·07*	*1·02*
4	1·09	1·19	—	1·15
	1·06	*1·15*	*1·07*	*1·00*
12	1·10	1·22	—	1·17
	1·07	*1·19*	*1·18*	*1·08*

is neither strong nor consistent. Tuscan, Solid-Straw Velvet and Yeoman increase their ear numbers at an equal rate and only Hunters lags slightly behind. This apparently is connected with the more favourable conditions for head formation in the second season, as illustrated by the large number of heads produced in the 12-in. spacings, *i.e.* under conditions which allow almost a maximum development of intrinsic productivity:

Heads per plant in 12-in. spacing.

	1929–30	1930–1
Tuscan	10·25	12·93
Hunters	8·60	10·84
Yeoman	6·91	10·96

Tuscan reacts to variation in spacing more consistently than Hunters and Yeoman with a regulation of head formation; whereas the latter varieties rely chiefly, though able to utilise this same mechanism under favourable circumstances, on the increase in yield per ear, when conditions do not allow of their optimal number of heads to be formed. It will further be seen that where ear weight is the chief adaptation, viz. in Hunters and Yeoman in 1929–30 and Hunters in 1930–1, the increase in grain weight seems to be as important as that in grain number; whereas in Tuscan, Solid-Straw Velvet and Yeoman (in 1930–1) the grain weight shows only small increases at wider spacing.

It appears that the chief regulatory mechanism of Tuscan and Solid-Straw Velvet is head formation, and that Hunters and Yeoman choose for yield adaptation between ear number and ear weight: when conditions are unfavourable for the former, the latter seems to take its place. Tuscan then relies on adaptation by head formation over a broader range of circumstances.

It seems possible, as is tentatively suggested in the introduction, to draw analogies between varying conditions of spacing and gradations of soil fertility: considerations of moisture and nourishment, and observations of plant growth tend to suggest a limited correspondence. Viewing the results of the spacing trials in this light it appears significant that those varieties which in some seasons at least rely on the relative development of head size for their chief adaptation, seem to suffer in their development in a densely crowded population. The yields per head in all spacings except the densest are considerably higher for Hunters and Yeoman than for Tuscan; but in the ½-in. spacing, both these varieties are equal or inferior in the first season, and Hunters in the second.

If conditions in a densely crowded population can, to a certain extent, be likened to those of lesser fertility and moisture, Hunters and Yeoman cannot be relied upon for adequate yields under these less favourable conditions, since it is just in these varieties that ear weight is largely used for adaptation. While the poor yield of Hunters and Yeoman under unfavourable conditions is well known in wheat-growing practice, the results seem to shed some light on the causative mechanisms, viz. relative development of ear number and size under varying environmental conditions.

C. CENSUS TRIALS.

Plant survival in the drilled trials is uniform in both seasons (Table XI, A) and, with the exception of Hunters in the second, presents no varietal differences. It ranges between 63 and 76 per cent. of the seeds germinated. Three months after sowing the number of seedlings is regularly the same as it was after germination, 4–5 weeks earlier. Repeated observations had shown that no seedlings had died before the first plant count. Practically all the losses in the population occur during the period between the second count (3 months after sowing) and harvest. This is contrary to repeated observations made in England (summarised by Engledow and Ramiah, 1930, pp. 312ff.), where the chief losses were suffered during the first 2 months, and, in the absence of parasitic damage, the population remained almost at an even level from that period till harvest.

Whilst this difference is clearly established, no conclusive evidence is available which may reveal its causes, or which might give a lead to avoiding the losses in the later stages of growth apparently regularly occurring under New Zealand conditions. But observation on field crops seems to suggest that foot-rot diseases of various kinds may possibly be responsible for the major part of the damage. Whilst under English conditions diseased plants would have little chance of surviving during late autumn, the milder New Zealand winter may give them a longer lease of life, only to be terminated by spring drought[1].

Tiller development in field crops (Table XI, B) follows closely on the lines observed in hand-sown crops. The characteristic varietal differences are clearly visible, though not nearly as marked as they are under the conditions of wider spacing and better cultivation as provided in dibbled plots. Similarly, tiller survival closely corresponds in both sets of trials, the characteristic order being maintained in both seasons, though the absolute values in the second are well below those reached in the chessboard.

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[1] The effect of foot-rot on wheat crops is being studied by Mr I. W. Blair of Canterbury Agricultural College.

Table XI. *Developmental observations and yield analysis in census trials.*

A. *Field germination and plant survival.*

Season	Date	Number of plants per 2 ft.			
		T.	S.S.V.	H.	Y.
1932–3	July 18 (7 weeks)	21·83	28·16	22·87	21·15
	Aug. 17 (11 weeks)	21·98	26·27	23·21	21·56
	Harvest	14·70	16·98	14·66	14·10
	Plant survival (%) (Aug. 17 to harvest)	66·9	64·6	63·2	65·4
		T.	Cr. 7	H.	J.
1933–4	July 7 (8 weeks)	21·39	25·30	21·76	20·13
	Aug. 8 (13 weeks)	21·37	25·62	21·61	20·66
	Harvest	13·88	17·58	16·47	13·87
	Plant survival (%) (Aug. 8 to harvest)	65·0	68·5	76·1	67·1

B. *Tiller development.*

Season	Date	Total number of tillers per 2 ft.				Number of tillers per plant			
		T.	S.S.V.	H.	Y.	T.	S.S.V.	H.	Y.
1932–3	Aug. 17	22·26	23·61	25·09	24·96	1·01	1·05	1·08	1·56
	,, 31	34·61	36·16	37·52	42·86	—	—	—	—
	Sept. 8	54·76	62·20	58·64	58·95	—	—	—	—
	,, 21	71·60	77·15	78·98	75·73	—	—	—	—
	Oct. 1	83·40	102·51	96·25	111·01	—	—	—	—
	,, 10	85·74	109·96	101·95	123·33	—	—	—	—
	Harvest (heads)	54·94	48·19	33·92	34·78	3·74	2·84	2·31	2·47
	Tiller survival (% (Oct. 10 to harvest)	64·0	43·9	33·3	28·2	—	—	—	—
		T.	Cr. 7	H.	J.	T.	Cr. 7	H.	J.
1933–4	Aug. 7	30·78	39·84	41·85	20·66	1·44	1·56	1·40	1·00
	,, 18	41·58	55·57	56·73	30·47	—	—	—	—
	,, 29	56·92	73·81	69·29	46·05	—	—	—	—
	Sept. 8	68·98	87·73	93·72	59·85	—	—	—	—
	,, 19	75·98	96·06	102·73	64·48	—	—	—	—
	Oct. 2	71·92	90·06	95·99	62·55	—	—	—	—
	Harvest (heads)	35·08	35·61	27·00	27·10	2·53	2·03	1·64	1·95
	Tiller survival (%) (Sept. 19 to harvest)	46·3	37·1	26·3	42·0	—	—	—	—

C. *Analysis of mature population.*

Season		T.	S.S.V.	H.	Y.
1932–3	Number of plants per 2 ft.	14·70 ± 0·63	16·64 ± 0·63	14·22 ± 0·63	14·10 ± 0·63
	Number of heads per 2 ft.	54·95 ± 2·68	47·23 ± 2·68	32·90 ± 2·68	34·78 ± 2·68
	Yield of grain per 2 ft. (gm.)	46·38 ± 0·81	41·69 ± 0·81	34·11 ± 0·81	37·47 ± 0·81
	Yield of grain per plant (gm.)	3·16	2·52	2·40	2·66
	Yield of grain per head (gm.)	0·84	0·88	1·04	1·08
	Number of heads per plant	3·74	2·86	2·31	2·47
	Number of grains per plant	71·69	74·17	58·15	67·24
	Number of grains per head	19·18	26·13	25·13	25·30
	Weight of 1000 grains (gm.)	44·06 ± 0·133	34·58 ± 0·133	42·94 ± 0·133	40·54 ± 0·133
		T.	Cr. 7	H.	J.
1933–4	Number of plants per 2 ft.	13·88 ± 0·50	17·58 ± 0·50	16·47 ± 0·50	13·87 ± 0·50
	Number of heads per 2 ft.	35·08 ± 1·49	35·61 ± 1·49	27·00 ± 0·51	27·10 ± 0·51
	Yield of grain per 2 ft. (gm.)	31·65 ± 1·35	30·64 ± 1·35	30·78 ± 0·44	22·04 ± 0·44
	Yield of grain per plant (gm.)	2·28	1·74	1·87	1·59
	Yield of grain per head (gm.)	0·90	0·86	1·14	0·81
	Number of heads per plant	2·53	2·03	1·64	1·95
	Number of grains per plant	43·51	39·05	38·83	32·36
	Number of grains per head	17·18	19·30	23·67	16·49
	Weight of 1000 grains (gm.)	52·40 ± 0·284	44·56 ± 0·284	48·16 ± 0·284	49·13 ± 0·284

The analysis of the mature population is given in Table XI, C. Standard errors were worked out only for those characteristics which could be determined directly, since owing to the variable nature of the number of plants per 2-ft. sample, the other characteristics possess no more than indicative value. The standard error was worked out by applying the analysis of variance. Considerable differences were found in the variances of different varieties, hence for some characteristics the varieties were grouped according to their variance.

A comparison between the order of varieties with regard to yield components, in chessboard and in census determinations, shows a high degree of correspondence. The only major discrepancy is that presented in 1932–3 by the better performance of Yeoman in the census trial chiefly due to the large ear size in the latter. From Table VI it becomes evident that Yeoman in that year yielded about 7 bushels per acre less than Hunters, in the same variety trial from which the census samples were obtained. This indicates faulty sampling. Hence the chessboard trial more truly represented the yield relations than did the census trial.

It may be concluded that a fairly close correspondence has been established between yields and yield components in hand-sown and drilled trials, and that therefore the former may be considered to render a true position of intervarietal relationships under the conditions of these trials.

Table XII. *Comparison of yield analyses on chessboard and census trials.*

1932–3	Chessboard				Census			
Yield per plant	T.	>S.S.V.	>H.	>Y.	T.	>Y.	>S.S.V.	>H.
Yield per head	H.	>S.S.V.	>Y.	=T.	Y.	=H.	>S.S.V.	=T.
Number of heads per plant	T.	>S.S.V.	>Y.	>H.	T.	>S.S.V.	>Y.	>H.
Number of grains per plant	S.S.V.	>T.	>H.	>Y.	S.S.V.	>T.	>Y.	>H.
Number of grains per head	S.S.V.	=H.	>Y.	>T.	S.S.V.	>H.	=Y.	>T.
Weight of 1000 grains	T.	>H.	>Y.	>S.S.V.	T.	>H.	>Y.	>S.S.V.
1933–4								
Yield per plant	T.	>Cr. 7	>H.	>J.	T.	>H.	>Cr. 7	>J.
Yield per head	H.	>T.	>J.	>Cr. 7	H.	>T.	=Cr. 7	=J.
Number of heads per plant	T.	>Cr. 7	>H.	>J.	T.	>Cr. 7	>H.	>J.
Number of grains per plant	T.	>Cr. 7	>H.	>J.	T.	>Cr. 7	>H.	>J.
Number of grains per head	H.	>J.	>T.	=Cr. 7	H.	>Cr. 7	>T.	>J.
Weight of 1000 grains	T.	>H.	=J.	=Cr. 7	T.	>J.	>H.	>Cr. 7

THE RELATIONS BETWEEN TILLERING, HEAD FORMATION AND YIELD. THE CRITICAL PERIOD OF TILLERING.

What for the business man is his balance sheet, is for the plant breeder the yield record and the yield analysis of his new productions. Both represent itemised accounts of returns. But just as the business man studies the origin and history of each item in order to draw general conclusions for future policy and organisation, so the plant breeder wishes to know the genesis of the yield characteristics exhibited by the mature population, the mechanisms responsible for the differences in yields and yield components, in order to correlate the elements of development with those of the ultimate product.

It is not intended to consider in this connection "ancillary yield characteristics" (Engledow and Ramiah, 1930), "Ertragsunterstützende Eigenschaften" (Heuser, 1928), such as winter hardiness, drought resistance, resistance to disease, lodging, etc., but rather certain phases in the development of the average normal plant, from germination to maturity.

Anticipating ideas recently expressed by Lyssenko (Maximov, 1934) and by Hudson (1934), Engledow and Wadham (1923–4) distinguished definite phases in the development of a graminaceous plant, such as germination, development of the first, second, ... leaf, of the first, second, ... tiller, heading, filling process, etc. The varietal differences in tillering modes, and particularly the time of incidence and the intensity of tillering, were associated with differences in yield, and were claimed as yield indices in plant-breeding work. These conclusions will be discussed below.

Boonstra has shown in Wilhelmina wheat that it is the uppermost leaves and the head which take the largest share in filling the grain. During the last weeks of the filling process the influence of the leaves seems to recede (Boonstra, 1929 *a*). Among four varieties of oats there were noticeable differences in the longevity of the leaves with a positive correlation between yield and longevity. This, in the author's opinion, may be utilised in plant breeding (Boonstra, 1929 *b*). An elaborate interpretation into varietal differences in root systems and their influence on yield was undertaken by Boonstra (1931). Differences in the root systems of cereal varieties, mainly with reference to the relative development of primary and secondary roots, have been reported by various investigators (Engledow and Wadham, 1923–4, and others), but no definite relations to yield characteristics have been established.

McMillan (1935) found in wheat positive correlations between seed weight and early growth with yield per plant, ear number and number of grains per ear. There is an indication of varietal differences, the correlation being stronger in one variety than in another.

In view of the large differences in the tillering modes exhibited by the common New Zealand wheat varieties, and also in view of the importance which was attributed to tillering in the Cambridge work, tillering, head formation, and tiller survival have been the chief points of interest in the developmental observations in this investigation.

In an analysis of the properties of the "critical period" of tillering, Engledow and Ramiah (1930) define it by the incidence of two phases, which never are separated by more than a few weeks:

(*a*) Only tillers formed before the critical period form ears.
(*b*) Up to the critical period spacing does not influence tillering.

The incidence of both phases is subject to considerable seasonal variations, and an early incidence is taken as an augury for a favourable yield owing to the long "physiological life" for the ear-bearing side tillers. The phase (*a*) shows little, if any, variation among the three varieties included in their experiments, whereas with respect to (*b*) there is a marked sequence in the order:

Yeoman > Squarehead's Master > Rivet.

The chief interest in the conception of the critical period is founded on the reported correlation between start and intensity of tillering on the one hand and yield on the other.

Since, under comparable conditions of spacing, there are practically no varietal differences in the time incidence of the critical period, "it follows for these three varieties, that the index to ear production per plant is rate of early tillering. General experience, especially in selecting segregate forms in hybridisation, suggests that this index has wide application among wheat, barley and oats, and is a valuable guide in breeding." It is further emphasised that in consequence of competition among plants and tillers, early tillering varieties, though producing a large number of ears, possess small heads. The range amongst the three varieties with respect to the characteristics involved is the following:

Start of tillering:	Yeoman	> Squarehead's Master > Rivet.
Number of ears:	Yeoman	> Squarehead's Master > Rivet.
Size of ears:	Rivet	> Squarehead's Master > Yeoman.

Special interest attaches to the lead which these conclusions seem to afford to practical plant breeding, both in the choice of parent forms, and particularly for the selection among hybrid lines.

An inspection of Table VIII of this paper shows that under New Zealand conditions and for the whole range of varieties included in these studies the phase (*b*) shows a smaller degree of varietal differentiation than obtains in the English experiments. The phase (*a*), on the other hand, almost a constant under English conditions, shows in New Zealand well-marked varietal differences. Up to 4 weeks elapsed between the critical periods of the most widely separated varieties in any one season (Table III; the position of the stars roughly indicates the incidence of the critical period).

Considering the two groups of high-yielding varieties, there is a tendency for an earlier start of tillering, and also one, though less marked, for an earlier critical period among the late-maturing varieties in comparison with the early ones. Within these groups, the varietal sequence in both these phenomena is fairly regular (Table XIII, A and B), and, comparing the two properties, more or less co-ordinated. Major leads among the early-maturing varieties, the rest being remarkably even, with a tendency for delay in Tuscan. Among the late-maturing varieties there are characteristic differences. The order of the incidence of tillering is well marked and practically without seasonal exceptions. A similar, though less differentiated, order exists for the critical period. In both properties Tuscan, entered for comparison, is markedly later than all the late varieties.

Table XIII. *Varietal sequence of tiller and ear development in chessboard trials.*

(> indicates "earlier" or "larger" than the following,
< "later" or "smaller" than the following.)

	Early varieties	Late varieties
A. Beginning of tillering		
1930–1	Maj. > D. = S.S.V. = T.	Y. > H. > Vic. > V. (= T.)
1931–2	Maj. > D. = S.S.V. > T.	Y. > S.M. > H. > Vic. > V. (> T.)
1932–3	S.S.V. = T. = Cr. 7	Y. = S.M. > H. > Vic. (> T.)
1933–4	T. = Cr. 7	Y. > H. (> T.)
B. Incidence of critical period		
1930–1	Maj. > D. = S.S.V. > T.	Y. = H. > Vic. > V. (> T.)
1931–2	Maj. > D. = S.S.V. = T.	Y. = S.M. = H. > Vic. = V. (= T.)
1932–3	S.S.V. = T. = Cr. 7	Y. = S.M. > H. = Vic. (> T.)
1933–4	T. = Cr. 7	Y. > H. (> T.)
C. Number of heads per plant		
1930–1	D. < Maj. < S.S.V. < T.	Vic. < H. < V. = Y. (< T.)
1931–2	Maj. < S.S.V. < D. < T.	S.M. < Vic. < H. = V. = Y. (< T.)
1932–3	S.S.V. < T. < Cr. 7	S.M. = Vic. < H. < Y. (< T.)
1933–4	Cr. 7 < T.	Y. < H. (< T.)
D. Yield per head		
1930–1	Maj. > D. = S.S.V. > T.	Vic. > Y. (> T.)
1931–2	D. > S.S.V. > T.	Vic. > S.M. = H. = V. > Y. (= T.)
1932–3	S.S.V. = T. = Cr. 7	Vic. > S.M. = H. > Y. (> T.)
1933–4	T. > Cr. 7	H. > Y. (< T.)

Turning to the association between tillering behaviour and yield, a comparison of Tables V and XI shows that the conclusions attained in

England cannot claim general validity for the range included in this investigation. It is evident that those varieties which are last to tiller and also last to enter the critical period, lead in head formation (Table XIII, A and B *v.* C), the most conspicuous difference being that between Tuscan on the one side and the late-maturing varieties on the other.

Taking the two groups separately it appears that in the early varieties the position is the reverse of that observed in England: early tillering seems to be associated with low head formation, late tillering with large head formation. Some of the late-maturing varieties, viz. Hunters, Yeoman and Victor, conform more or less to the rule established in England; but Velvet, one of the last to tiller, is ahead of Hunters in head formation, and Squarehead's Master, with Yeoman, the first to reach the critical period, closes the list for number of heads. The inverse relation between ear number and ear size (Table XIII, C *v.* D) is only too apparent, though obviously detached from the tillering mechanism which in the English work seemed to cause this negative correlation.

Referring in particular to those varieties which were included in New Zealand as well as in England, viz. Squarehead's Master and Yeoman, their relations are very similar under both sets of conditions:

	England	New Zealand
Start of tillering	Y. > S.M.	Y. > S.M.
Critical period (phase (*a*))	Y. = S.M.	Y. = S.M.
Number of heads per plant	Y. > S.M.	Y. > S.M.
Yield per head	Y. < S.M.	Y. < S.M.

No certain inference can therefore be drawn whether under English conditions the range of varieties tested in New Zealand would conform to the principles established in the Cambridge trials. But it can be concluded that under New Zealand conditions these principles, though valid for some forms, have no general applicability. either when taking a wide range of diverse forms as a whole, or when confining the comparison to high-yielding English varieties of a type related to the forms used at Cambridge.

In this connection may be recorded another difference in the mechanisms of tiller and head formation under English and New Zealand conditions. In the Cambridge trials both yield per ear and weight of 1000 grains were found to increase steadily with an increasing number of heads (Engledow and Wadham, 1923–4; Engledow, 1925; Engledow and Ramiah, 1930). In consequence, plants with two heads return in excess of twice the yield of one-head plants, etc. "Thus tillering, with its culmination in ear production, is doubly an index to plant development." By adding to the importance of high-tillering forms, this raises a question of considerable practical importance.

An increase in yield per panicle with an increasing number of panicles per plant was found by Boonstra (1929 *a*) in an experiment with oats. It is interesting to note that this was restricted to the three western European varieties, whereas in an Algerian variety, adapted to primitive conditions, this increase was altogether absent.

It has been reported previously that in the first experimental season in New Zealand no confirmation for these observations was found (Frankel, 1931, p. 57 f.). Since then similar results have been obtained annually. There is little or no increase in the average ear size with increasing ear number, the only exception being the difference between one-ear and two-ear plants, which—owing to one-ear plants mostly being weak or diseased—generally is considerable. But among ear-number classes from two ears upwards there are no such marked differences in ear or grain size as have been found in England. This may be exemplified by the means for the "early" and "late" varieties in 1931–2, and also by two of the varieties used in Cambridge.

Table XIV. *Yield per ear in ear-number classes.*

"Four ears" = 100.

	1 ear	2 ears	3 ears	4 ears	5 ears	6 ears	7 ears
Early varieties	76·1	92·7	95·5	100	101·5	95·2	95·9
Late varieties	80·1	97·0	99·5	100	101·0	98·7	—
Squarehead's Master	83·8	109·1	104·0	100	104·0	85·9	—
Yeoman	84·2	93·7	98·9	100	96·8	98·9	—

If these data permit of any general conclusions on the ecological differences between the two habitats, it would appear that under the circumstances of the New Zealand experiments, conditions did not favour the development of ear and grain to the same extent as they did in England; there seems to be a higher elasticity of ear development under English conditions. This is further exemplified by a comparison of the ear size of identical varieties grown under equal conditions of spacing in England and in New Zealand (Table XV), demonstrating that ear size is markedly larger in England than in New Zealand.

It seems that the cause for this difference may be sought in the different conditions of soil and management.

Engledow and Ramiah (1930) conclude that the limitation of ear development can be overcome only by high farming. It is now suggested that wheat growing in New Zealand does not generally provide conditions sufficiently favourable for a full development of ear size. This conclusion seems to provide an explanation for the fact that the modern high-yielding varieties of western and north-western Europe, with their enhanced reliance on ear development, have in New Zealand proved successful only under the most favourable conditions of soil and management; and that an old English variety, Hunters, now extinct in its country of origin, has been able to conquer and to hold the more favourable localities of New Zealand against the repeated assaults of a host of modern rivals—all of them better equipped to utilise higher farming conditions, but none as able as Hunters to thrive at the level of soil and cultivation obtaining in New Zealand wheat growing[1].

Table XV. *Ear size of Squarehead's Master and Yeoman grown at Cambridge and at Lincoln for a number of seasons.*

Spacings 6 × 2 in. (yield per ear, in gm.).

	England (Engledow and Ramiah, 1930)		New Zealand	
	S.M.	Y.	S.M.	Y.
	1·72	1·34	—	1·28
	1·16	0·89	—	1·08
	2·14	1·69	1·02	0·93
	1·92	1·55	1·22	1·03
	—	—	—	1·20
Mean	1·72	1·37	1·12	1·10

Turning now to the striking difference in tiller and head formation between the two most important New Zealand varieties, Tuscan and Hunters, their different modes of tiller survival may supply an explanation for their ecological characteristics.

It has been shown above that in New Zealand wide differences are found in the rates of tiller survival of various forms of wheat; that the erect, sparsely tillering types are in general vastly superior in tiller survival to the prostrate, profusely tillering forms. In countries with a more equable moisture supply, such differences seem to be absent, or at least they are far less remarkable. Heuser and Boekholt (1931) experimenting in north-eastern Germany, grouped twelve European varieties into three classes, according to juvenile growth type and tillering intensity.

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[1] The fact that average yields per acre in England and New Zealand are almost identical, does not seem to contradict the above statement. If conditions were favourable to a cultivation of the modern "intensive culture" varieties, yields in New Zealand probably would be higher than they are at present.

Group	Juvenile growth type	Average number of tillers per plot	Average crop density at harvest	Average tiller survival %
I	Mainly prostrate	2526	444	17·9
II	Mainly intermediate	2159	370	16·8
III	Erect	1623	321	19·3

Tiller formation is similar to that found in New Zealand in forms with similar types of juvenile growth. But head formation and, consequently, tiller survival are closely linked up with vigour of tillering. Hence tiller survival is equal in all three groups, whereas in New Zealand the tiller survival in corresponding groups ranges between 20 per cent. in prostrate and 65 per cent. in erect forms (Table IV above).

Forster and Vasey (1931), on the other hand, have illustrated the high importance of the rate of "percentage survival" for the dry conditions of Victoria: "...'Percentage Survival' of tillers to heads has been of the greatest importance in indicating drought resistance. All the outstanding new varieties in general cultivation show a marked increase in the value of this factor." Although the climatic conditions of the wheat-growing areas of New Zealand are more closely similar to those of England than to those of Australia, dry spring weather in Canterbury (New Zealand) is not at all uncommon. In fact, the supply of moisture during spring is held to be the main limiting factor for wheat yields in Canterbury (Hilgendorf, 1935)[1].

From the early days of colonisation in New Zealand, Mediterranean wheats of a type resembling that of Solid-Straw Tuscan have been used extensively, gradually extending their area of cultivation at the expense of English varieties, until to-day the latter are restricted to about 20 per cent. of the area in wheat, comprising the high-fertility districts. These earlier forms were not nearly as resistant to shedding as their successor, Solid-Straw Tuscan. Neither resistance to disease or to lodging, nor earliness played any considerable role. It may be suggested as the most

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[1] Dr F. W. Hilgendorf, Director, Wheat Research Institute, added the following comment:

The chief point of difference between England and Canterbury (New Zealand) is that evaporation in New Zealand is so much greater than in England, that the soil conditions approximate more to Australia's than to England's:

	Rainfall in.	Evaporation from free water surface in.
Essex	25	20
Lincoln (New Zealand)	25	34

likely explanation for their spreading that their high tiller survival and head formation were the characteristics fitting them for the task of "all round wheats": high tiller survival, effecting an economical use of scanty resources of moisture and nourishment; and high ear formation, enabling the full utilisation of a more favourable environment. Both properties combined may be adduced as the cause of the striking adaptability of Tuscan, exemplified by its behaviour in the spacing trials, and demonstrated by its almost universal distribution.

CONCLUSIONS.

Plant mortality, both in hand-sown plots and under field conditions, does not regularly exhibit varietal differences. In the former it is generally low, the losses being about equally distributed over the whole of the vegetation period. Only in one season were there marked varietal differences, all except Tuscan suffering more or less heavily. Spacing does not seem to influence the rate of survival. In field crops the proportion of surviving plants is similar throughout the seasons and for all varieties. Whereas in England the majority of plant deaths occur within the first 2 months after sowing, in New Zealand the population numbers show no decrease until 3 months after sowing, all the losses occurring in later stages of development. This, it is suggested, may be due to a differential length of life of plants injured by foot-rot organisms. Plant mortality appears to be one of the major limiting factors for wheat yields in New Zealand. There is an obvious need for investigation of its causes and of possible remedies.

Tiller development exhibits marked and characteristic varietal differences in the incidence of start of tillering and of the critical period, in the relative proportion of tillering before and after the critical period, and in the total tiller production. An early start is associated with profuse tillering and *vice versa.* Contrary to conclusions reached in England early tillering is neither an index of head formation nor of plant yield within a wider range of forms; but for the two varieties included both in New Zealand and in England, these conclusions are valid under both sets of conditions.

Tiller survival[1]—the proportion of tillers maturing ears and its relations to the productivity of the mature plant must be interpreted from the varietal and from the environmental point of view. In a trial in Germany it was constant for varieties including a wide range of growth forms and tillering modes. Head formation depended on the varietal rate of tiller production. In Australia, tillering appears to be an inverse index of yield: low tillering and high tiller survival are of chief importance.

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[1] The physiological interrelations of the tillers of a graminaceous plant are still not sufficiently understood. It is generally accepted that an excess of tillers above those which form ears is superfluous or even undesirable, "since they originally start by forming ear primordia which ultimately die off, making no addition to the final yield of the plant"

In England, early and high tillering are the chief indices of large number of heads, and through this of high yielding capacity. In New Zealand, the variety which covers more than 70 per cent. of the wheat land combines low tillering with the largest number of heads. As an expression of adaptation to occasional drought periods, combined with a faculty for utilising highly favourable conditions, these characteristics are interpreted as being responsible for the "all-round" character of Tuscan under New Zealand conditions. Varieties relying on a large yield per head are unsuitable over the larger part of the Canterbury plains: English as well as late-maturing Australian varieties, both characterised by large heads, though widely different in growth type and growth rhythm, fail everywhere except under very favourable circumstances. But even in those districts not one of the modern western European varieties—though scores of them have been introduced—has displaced Hunters, a variety which in England has been ousted by forms better able to take advantage of the superior farming methods of to-day.

Head formation and ear size; the grouping of varieties.

The main varieties of New Zealand can be grouped into (1) "all round" types, (2) high-farming types—"high-farming" in the sense of superior classes of land—and (3) spring wheat types. The first includes Tuscan, Cross 7, and Solid-Straw Velvet; the second, Hunters, Velvet, Yeoman, Victor, Dreadnought and Major; the last, Jumbuck, Garnet and Marquis. A number of varieties not cultivated in New Zealand, but included in these studies, fall into either the second (Squarehead's Master) or third group (Reward, White Fife, Red Fife, Tenmarq).

The first group is characterised by sparse tillering, high tiller survival, large number of ears, small to intermediate ear size, and large to inter-

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(Hudson, 1934). Whether this interpretation is entirely correct is still open to doubt. Recent work by Smith (1933) shows that at least in some circumstances exchange between tillers takes place, though probably this is unimportant under normal conditions. In unpublished work by the present author on variegated barley it has been found that in striped plants tillers nearly devoid of chlorophyll matured grains, whereas similar plants, unattached to other units richer in chlorophyll, died within a few weeks after germination.

mediate grain; the second by profuse to intermediate tillering, low tiller survival, small to intermediate number of ears, and large to intermediate grain. Members of the first group are more adaptable to variation in spacing than members of the second. In the third group, as might be expected from its heterogeneous composition, various types are included, reflecting the ecological conditions of their homelands. Hence Jumbuck, an Australian variety, is characterised by sparse tillering, high tiller survival and large grain. All the types of group (3), with the exception of Jumbuck, have in common small grain, which is their chief limiting yield factor.

Intergroup correlations of yield components and yield would serve no useful purpose. Botanically and agronomically these groups are too widely separated. Within the groups the numbers are too small to facilitate statistical treatment. But this does not prevent intragroup comparisons as have been given for Solid-Straw Velvet and Tuscan, and for Hunters, Squarehead's Master and Yeoman.

The significance of analytical generalisations.

Important as the interrelations between tillering mode, head formation, and yield appear to be in any individual case, it seems impossible to draw conclusions of a general nature. Consider, for example, large number of heads as a yield index: in England it has been interpreted as characteristic of a form for high-farming conditions, the all-round type being inferior in the rate of head formation; in New Zealand, the all-round form is taken to owe its adaptability to the elasticity of head formation, the "high-farming" form on the other hand as inferior in this respect.

This does not mean that conclusions reached in one part of the world are of no interest to the rest. On the contrary, comparisons between results obtained in various countries are of the highest value in forming conclusions in any of them, particularly if the same varieties have been used. But it is obvious that the validity of these conclusions is limited to the conditions under which they were obtained.

In any individual case, analytical results are doubtless of an eminent value. If taken in combination with information obtainable from varietal distribution, farming practice, variety trials, ecological observations, soil surveys, they can help materially, to unravel the yield mechanisms of crop varieties. Naturally, as has also been observed by Smith (unpublished) and as can be illustrated from data in Table V of this paper, equal plant yields can be obtained in various ways. But the yield characteristics of established varieties—established usually after decades of large-range trials in the hands of that most critical experimenter, the practical farmer—will serve as an obvious guide towards further improvement.

ACKNOWLEDGMENTS.

Every acknowledgment is due to Prof. F. L. Engledow, who with his collaborators clarified the ideas and devised the methods of a yield analysis in cereals.

The author wishes to acknowledge his indebtedness to Dr F. W. Hilgendorf, Director, Wheat Research Institute, for his interest and encouragement during the whole of the experimental work, and for many helpful suggestions and criticisms during the preparation of the manuscript. Mr F. R. Callaghan, of the Department of Scientific and Industrial Research, and Mr A. W. Hudson, of Massey Agricultural College, read the manuscript and offered helpful criticisms.

The author is particularly grateful to Mr H. F. Smith, Research Officer, Commonwealth Department of Scientific and Industrial Research, who lent valuable advice and assistance in connection with the statistical elaboration of the census results. He also made available the manuscript of his latest paper on yield analysis.

Mr C. V. Woodfield, of the staff of the Wheat Research Institute, has been in charge of all sowing and harvesting operations.

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(*Received July 4th*, 1935.)

A NOTE ON THE INFLUENCE OF RAINFALL ON THE YIELD OF CEREALS IN RELATION TO MANURIAL TREATMENT.

By W. G. COCHRAN, B.A.

(*Statistical Department, Rothamsted Experimental Station, Harpenden.*)

(With One Text-figure.)

INTRODUCTION.

EXPERIMENTS on the continuous growth of wheat and barley under the same manurial condition year after year have been in progress at Rothamsted, on Broadbalk and Hoosfields respectively, since 1844, and at Woburn, on Stackyard field, since 1877. Each field contained a number of plots receiving different manurial treatments. The systems of manuring were not identical in the four series, but they had certain common features, each being designed to cover a certain range of nitrogenous and mineral manuring.

In view of the relatively long series of years available, these experiments provide valuable data for the quantitative study of the effect of meteorological factors on the yield of wheat and barley. Progress in agricultural meteorology has, in fact, been greatly hampered by the scarcity of data sufficiently long that valid conclusions might be drawn from them. In 1923 R. A. Fisher(1) made an examination of the effect of rainfall on the annual yields of wheat on thirteen plots at Broadbalk which had received continuous manuring over the period 1852–1918. The other three series of data were later examined by the same method for rainfall effects, the barley yields from Hoosfield by J. Wishart and W. A. Mackenzie(2), and the wheat and barley yields from Woburn by A. M. Webster. Thirteen of the Hoos plots were chosen for study, the period being 1853–1921; at Woburn ten plots were taken for each crop over the much shorter period 1877–1906. The same method of analysis was also applied by R. J. Kalamkar(3) to the continuous experiments with mangolds at Rothamsted.

Probably the most striking of the manurial effects in these experiments, apart from that on mean yields, was the difference which they produced in the rates of deterioration of the yields throughout the experimental period. The primary object of the present note, however, is to show from the data at Rothamsted and Woburn that there is a close relation between manurial treatment and the variation in yield from year to year even after eliminating the effect on variation of such factors as deterioration of the soil. In order to appreciate the bearing of this point on previous studies of the Rothamsted and Woburn experiments, it is necessary to recapitulate briefly some of the methods and results in the papers mentioned above, beginning with Fisher's examination (1) of the effect of rainfall on wheat at Broadbalk.

Before investigating the rainfall effect it was necessary to eliminate from the series of annual yields the slow changes throughout the period, as these were considered to be due to deterioration of the soil, influence of weeds, etc., but not to any marked extent to the effects of the weather. To do this Fisher fitted a smooth curve, a polynomial of the fifth degree, to the annual yield of each of the thirteen plots and used the deviation in yield each year from the value given by the curve for the examination of rainfall effects. He also developed a technique for finding the linear regression of the annual yields on the rainfall throughout the year which leads to curves showing the change in yield expected from an inch of rain above the average falling at any time of year. A separate curve was fitted to each of the thirteen plots.

The curves were definitely significant on most of the plots, and on four plots more than 30 per cent. of the total variation in yield from year to year could be ascribed to rainfall effects. Further, when considered in relation to the manurial treatments of the different plots, they were capable of a simple and consistent interpretation. To quote Fisher (*loc. cit.* p. 125): "An examination of these diagrams shows how intimately the response of the crop to weather is connected with the manurial conditions of the soil. Classing the plots solely by inspection of the curves of response to rainfall we shall put together every case in which the manurial treatment is alike, and indeed the whole series of curves arrange themselves in sequence of order of increasing abundance of nitrogenous fertilisers."

In marked contrast to the results obtained on Broadbalk, the regression function was not nearly significant on any of the thirty-three plots at Rothamsted on Hoosfield and at Woburn. At Woburn the number of years involved in the test was unfortunately rather small, but for each crop three plots received continuous manuring for 50 years, and the rainfall curves for these showed the same lack of significance as the 30-year curves.

While not individually significant, the curves in each series grouped themselves by position and shape according to the type of manurial treatment they received just as on Broadbalk. This at first sight seemed considerably to increase the confidence to be put in their interpretation, for if the curves did not represent a real effect their grouping might be expected to be entirely fortuitous. Further, it was possible that while none of the individual curves was significant, the differences between the curves on two plots with different types of manurial treatment might be significant. At Woburn, the significance of the difference between the rainfall curves was tested for the barley plots which received mineral manures and mineral manures plus nitrate of soda respectively. The method used was to fit a rainfall curve to the differences between the deviations from the long-term polynomials of the two plots for each year, in exactly the same way as rainfall curves were fitted to the deviations of the individual plots. The curve can, of course, be obtained by taking at every point the difference between the two rainfall curves already fitted. A test of the significance of this curve against the deviations of the differences from the curve is a test of the difference between the curves for the two plots. This test is not the only one which suggests itself, and a discussion of its appropriateness will be given after the main results of the paper.

The actual difference tested at Woburn showed no sign of significance, and it is expected that if any other two plots had been taken the differences would not have approached significance either. In view of this and of the lack of significance of the rainfall curves themselves, it cannot be asserted with any confidence that they represent a real effect at all, despite the apparent grouping.

Considerable further search was made for particular rainfall variates which might significantly affect the yields of wheat and barley at Woburn, account being taken of quadratic and cubic terms and of variates related to the sowing date instead of to the calendar date. A number of possible factors were examined, but for wheat not one was found which had a significant influence. For barley, however, the combination March–April rainfall, its square and cube, and rainfall 60–90 days after sowing and its square gave regression curves significant at the 5 per cent. point on six of the ten plots.

THE ASSOCIATION OF SEASONAL VARIATIONS IN YIELD WITH MANURIAL TREATMENT.

It has already been mentioned that, before examining regressions on rainfall, the effects of deterioration and slow changes were eliminated from yield by fitting a smooth curve to the annual yields. The deviations of the actual yield from the value given by the curve are considered to represent that part of yield which is due to the effects of the season. High correlations are to be anticipated, and are actually found to exist between the deviations for different plots on the same field, for in a favourable season yields are in general higher than usual whatever the system of manuring. It was noticed by Yates, however, that the correlations between deviations appear to be much higher for plots receiving the same type of manuring than for those receiving different types. In other words, the deviations show the same natural grouping according to manurial treatment which has been evident in all the rainfall curves discussed above.

The object of this note is to draw attention to this fact and to show by a numerical test that the grouping is statistically significant. The circumstance itself is of considerable importance in the study of the effects of weather on yield, particularly in view of the comparative failure of the investigations at Woburn to discover any clear weather influences. For it points definitely to the existence of some combination of factors, possibly meteorological, whose influence on yield is closely related to the manurial treatment of the soil. Incidentally, it explains why the rainfall curves at Woburn grouped themselves according to manurial treatment, although there was no clear evidence of rainfall effect of the type examined. The grouping, if not a true rainfall effect, was apparently simply a reflection of that in the deviations to which the curves were fitted.

The search for such a combination of variates by the usual regression method would involve in any particular case tests on two points: (1) the significance of the regressions, (2) whether the high correlations between plots receiving the same type of manure remained after taking account of the effect of the particular factors studied.

It seems clear that the rainfall curves for wheat and barley at Woburn, as found by Fisher's method, satisfy neither of the two tests: they are not nearly significant, and it is quite certain that the grouping would remain if deviations from the rainfall curves were taken instead of deviations from the long-term polynomials. With wheat, indeed, since no meteorological factor was found which significantly affected yields, the precise explanation of the grouping has not been found. With barley, however, it is worth while applying the second test to the factors March–April rainfall, its square and cube and rainfall 60–90 days after sowing and its square, in view of the fact that they were found to have a significant effect on yield.

The regressions on March–April rainfall, etc., were worked out for the period 1878–1906 on deviations from six-year means instead of from the long-term polynomials. The former will therefore also be used in testing the significance of the grouping before taking account of rainfall effects, though it would show equally well in the deviations from the long-term polynomials. Six plots were taken, and these were found to be grouped according to manurial treatment into three groups as follows: (1) and (7) (no manure) and (4) (minerals); (3) (nitrate of soda) and (6) (nitrate of soda and minerals); (11*b*) (farmyard manure). Let (1), (7), (4), (3), (6) and (11*b*) denote the deviations of the corresponding plots from the six-year means in any year, so that $\Sigma\{(1)-(7)\}^2$, for instance, is the sum of squares of the differences between the deviations for plot 1 and those for plot 7, over the 29 years 1878–1906. This sum has 24 degrees of freedom. The differences in degree of association will be shown by comparing the sums $\frac{1}{2}\Sigma\{(1)-(7)\}^2$, $\frac{1}{6}\Sigma\{(1)+(7)-2(4)\}^2$, $\frac{1}{2}\Sigma\{(3)-(6)\}^2$, $\frac{1}{6}\Sigma\{(3)+(6)-2(11b)\}^2$ and $\frac{1}{6}\Sigma\{(1)+(7)+(4)-(3)-(6)-(11b)\}^2$, each of which has 24 degrees of freedom. The theory of the method may be explained briefly for the comparison of

$$\Sigma_1=\tfrac{1}{2}\Sigma\{(1)-(7)\}^2 \quad\text{and}\quad \Sigma_2=\tfrac{1}{6}\Sigma\{(1)+(7)-2(4)\}^2.$$

Examination of $\Sigma(1)^2$, $\Sigma(7)^2$ and $\Sigma(4)^2$ shows that it is legitimate to assume that (1), (7) and (4) have the same standard deviation. If the correlation between any pair of (1), (7) and (4) is the same, it is easy to see that $\frac{1}{24}\Sigma_1$ and $\frac{1}{24}\Sigma_2$ will be independent estimates of the same variance. If, on the other hand, (1) and (7) agree more closely in their variation from year to year than does either with (4), Σ_2 may be expected to be larger than Σ_1. Thus a test of the ratio Σ_2/Σ_1 by Fisher's z distribution is a test whether the yield of the minerals plot 4 shows a difference in its manner of variation from year to year from that of plots 1 and 7 which receive no manures. Similarly any other pair of the seven sums given above may be tested by the z test. The test assumes that (1), (7), (4), (3), (6) and (11*b*) have all the same standard deviation. This is not quite the case. For both wheat and barley the standard deviations of

(1), (7) and (4) may be taken to be the same, and so may those of (3), (6) and (11*b*), but the latter are much higher than the former. For barley, for instance, the mean squares $\frac{1}{24}\Sigma\{(1)\}^2$ etc., are: plot 1, 43·5; plot 7, 38·6; plot 4, 59·3; plot 3, 100·3; plot 6, 95·0; plot 11*b*, 107·6. These estimates were pooled, and the variance of (1), (7) and (4) was taken to be 47·1 and that of (3), (6) and (11*b*) 101·0. To make the standard deviations equal, each yield was divided by its standard deviation, thus reducing (1), (7), (4), (3), (6) and (11*b*) to a set of quantities each having unit standard deviation. The mean squares obtained from the deviations from six-year means for wheat and barley are shown below.

		Mean squares (24 degrees of freedom)	
	Manurial comparisons	Barley	Wheat
Within groups:			
(1) − (7)	Between unmanured plots	0·101	0·134
(1) + (7) − 2 (4)	No manure *v.* minerals	0·107	0·145
(3) − (6)	N/S *v.* N/S + minerals	0·061	0·131
Between groups:			
(3) + (6) − 2 (11*b*)	N/S *v.* F.Y.M.	0·228	0·307
(1) + (4) + (7) − (3) − (6) − (11*b*)	No N *v.* N	0·372	0·414

The first point which the analysis reveals is the high correlations which exist between the deviations for all plots compared. For owing to the method of weighting adopted, the mean squares should, in the absence of any correlation between the plots compared, have an average value 1, and when compared with 1 by the χ^2 distribution they are all significantly lower at the 1 per cent. point.

As regards the comparison of mean squares between and within groups, the results are similar for the two crops. The three sets of 24 degrees of freedom representing the variance within groups agree satisfactorily, whereas the two variances between groups are both significantly higher than any of the variances within groups. This demonstrates clearly the grouping of the deviations.

The next step is to test whether the grouping persists for barley after eliminating the effect of the significant weather variates which were found, viz. March–April rainfall, its square and cube, and rainfall 60–90 days after sowing and its square. To make the test, exactly the same procedure is carried out on deviations from the rainfall regression curves. In this case the mean squares $\frac{1}{19}\Sigma(1)^2$, etc., were (1) 35·5, (7) 25·4, (4) 38·4, (3) 67·1, (6) 58·8, (11*b*) 78·8, so that the estimated variances for the two groups were 33·1 and 68·2 respectively. Each yield was divided by the corresponding estimate of its standard error as before. The results in this case were:

	Mean squares (19 degrees of freedom)
Within groups:	
(1) − (7)	0·163
(1) + (7) − 2 (4)	0·118
(3) − (6)	0·081
Between groups:	
(3) + (6) − 2 (11 *b*)	0·265
(1) + (4) + (7) − (3) − (6) − (11 *b*)	0·602

If the high correlations between the deviations from six-year means of plots receiving the same type of manuring were entirely due to the different effects of the weather variates we are considering, the mean squares between and within groups would be of the same size. The results above, however, are not substantially different from those obtained for barley before eliminating the effects of March–April rainfall, etc. There is some sign of a removal of part of the difference between the nitrate of soda and farmyard manure plots by the rainfall regressions, since the mean square of [(3) + (6) − 2(11*b*)] is no longer significantly higher than that of [(1) − (7)] or [(1) + (7) − 2(4)]. On the other hand, it is still significantly higher than [(3) − (6)], with which it is more strictly comparable; and the mean square representing the difference between the no-nitrogen groups is still significantly above all mean squares within groups. It will be noticed that all mean squares rose after taking account of the rainfall effect. Such a rise means that the correlation between the sets of plots which we are comparing is less than before. This is what we should expect, since general similarity in rainfall response is presumably one of the causes of the high correlations which exist between all the plots. If the grouping of plots were due in part to a differential effect of rainfall, however, the percentage increase in mean square would be greater for the comparisons within groups than for those between groups.

The general conclusion from the above investigation is that for barley at Woburn, as for wheat, the explanation of the grouping has not been found. It must be remembered, however, that while the statistical methods employed in the study of rainfall effects constitute an advance on any used in similar studies previous to Fisher's paper (1), the particular types of weather effect studied are extremely simple and crude from the point of view of plant growth. It seems likely that little further progress towards the elucidation of weather effects on the yields at Woburn will be made by the statistician alone. For any further work on the present lines must resolve itself into an examination of correlations suggested by the data themselves. This method must be used with caution, particularly in view of the small number of years available for study, and it is unlikely to lead to fruitful results. The data will, however, remain of value for testing any theories which may arise as to the quantitative influence of weather on yields.

It is of interest to apply the two tests made on the Woburn data to some of the rainfall regressions found by Fisher's methods on Broadbalk. Here, as has been said, the rainfall curves were definitely significant and the differences between curves of plots in different manurial groups appear to be also significant. There seems, therefore, reason to expect that some of the difference between plots with different manurial treatments will disappear when the difference in rainfall effects is eliminated. Five plots were chosen which can be divided into three groups as follows:

(3) (no manure) and (5) (minerals).

(10) (ammonium salts, 200 lb. per acre) and (11) (ammonium salts and superphosphate).

(2*b*) (farmyard manure, 14 tons per acre).

The deviations on which the calculations are made are in this case measured from the long-term polynomials. As with Woburn, the variances of (3) and (5), and likewise those of (10) and (11), may be assumed the same, but that of (2*b*) differs from both of the others and the estimates, based on 54 and 48 degrees of freedom before and after eliminating rainfall effects respectively, have to be used as the true standard deviation. It may be mentioned that, while making the necessary calculations, the differences in rainfall curves corresponding to the two comparisons between groups made below were tested by the test mentioned above, both being found to be significant. Thus there is no doubt that on Broadbalk the differences in rainfall response between plots with different manuring are real. The mean squares obtained are shown below.

		Mean square of deviations from	
	Manurial comparisons	Polynomials (54 degrees of freedom)	Rainfall curves (48 degrees of freedom)
Within groups:			
(3) − (5)	No manure *v.* minerals	0·091	0·126
(10 − (11)	Ammonium salts *v.* ammonium salts + superphosphate	0·150	0·191
Between groups:			
(3) + (5) − (10) − (11)	No N *v.* ammonium salts	0·607	0·709
(3) + (5) + (10) + (11) − 4 (2*b*)	F.Y.M. *v.* rest	0·448	0·483

The mean square has again risen in all cases after eliminating rainfall effect. Further, the percentage increases are considerably greater for the comparisons within groups than for those between groups, indicating that part of the grouping is due to a differential response to rainfall. The correlations within groups, however, remain significantly higher than those between groups after eliminating the linear effect of rainfall, just as for Woburn barley. Thus on Broadbalk also the close relation between seasonal variation in yield and manurial treatment remains after eliminating that part of the variation, in some cases quite considerable, which may be ascribed to the linear effect of rainfall.

It is of course to be expected that when the same manuring is applied year after year to a plot it will have its effect on the way in which the crop responds to the varying succession of seasons; and as the principal result of this paper is merely a confirmation of this expectation it might not, if standing by itself, be considered of much interest. The interest of the result lies in its bearing on the interpretation of the studies of rainfall effect on Hoosfield, Rothamsted and on Stackyard, Woburn, for it shows why the rainfall curves could be classed according to the type of manurial treatment despite their lack of significance, and suggests that the failure to obtain significant effects of rainfall was due to the complexity and not to the relative unimportance of rainfall effect.

TESTS OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN RAINFALL CURVES.

It has been mentioned above that one method of testing the significance of the difference between the rainfall curves of two plots is to test in the usual way the significance of the rainfall curve fitted to their difference. Given replicates of the same treatment on the same field, a second test suggests itself. In this we fit a rainfall curve to each replicate, and test the difference between the mean curves for each treatment against the difference between the rainfall curves for replicates of the same treatment. In this case, of course, the first test can be made on the mean curves for the two treatments.

The two methods are not, in general, equivalent. An obvious difference between them is that a significant difference found by the first method is not necessarily attributable to the different manurial treatments, but may be due to soil heterogeneity, since no account has been taken in the test of the differences between replicates. There is, however, another important difference between the tests which is best illustrated by examining in more detail a simple particular case.

Suppose that we are examining the linear regression of yield on total rainfall for a particular treatment of which we have several replicates, and that the true response curve of yield to rainfall for one of the replicates is as shown in Fig. 1. We may assume further for simplicity that the points to which the regression is fitted lie closely along the true response curve. If the number of years taken is sufficiently large that the total range of variation of rainfall is reasonably well sampled, the regression line obtained for the mean yield might be somewhat as shown by the line R_1 in the figure. Let its slope be b, with an estimated standard error s_1, obtained from the deviations of the yields from the regression line. Then since s_1 includes contributions from the deviations of the annual yields from the line R_1 in an approximately random sample of rainfall totals, the limits $(b \pm 2s_1)$ may be expected to include the slope which would have been obtained had the rainfall totals been any other random sample of the same size from the whole population of rainfall totals. As the line R_1 has been drawn, its gradient would probably not be significant on the first test, and this may be said to represent the fact that with another sample of rainfall totals, slightly wetter than the one obtained, the gradient would be very small and might even be reversed as shown by the line R_2. The slope of R_2 would probably not be significant either on the first test. Since there are replicates, we can here apply the second test also by fitting a regression line on rainfall to each replicate and testing the deviation of the average gradient from zero by comparison with the differences between the gradients obtained for the various replicates. Each of these gradients, is, however, obtained from

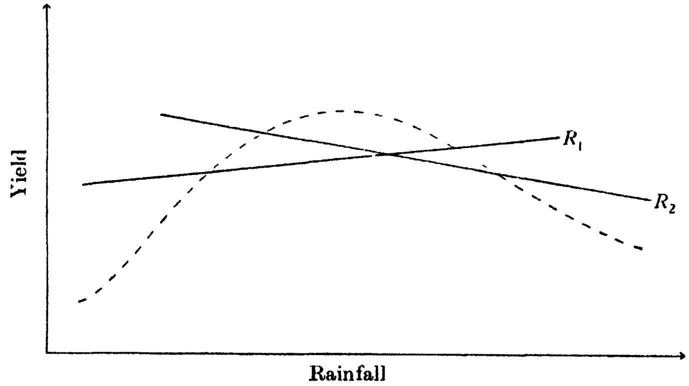

Fig. 1.

the same set of rainfall totals, and if the difference in rainfall response due to soil heterogeneity is small, they would probably all slope in the same direction as R_1. Thus R_1 might well be significant on the second test. Similarly, with the set of rainfall totals which lead to R_2, it might also be found significant on the second test, though the gradients of R_1 and R_2 are opposite in sign. In fact, if s_2 is the estimated standard error of b as found by the second test, the limits $(b \pm 2s_2)$ give no indication of the gradient which would have been obtained had the set of rainfall totals been different. Any deductions made from the second test are, therefore, valid only for the particular set of rainfall totals which happened to be present in the data.

The question as to which is the better test does not really arise. What is important is to realise the correct interpretation to be put on the results of each test, and for this the differences between the tests should be kept in mind. If there is no real difference in rainfall curves due to soil heterogeneity, it is clear that, when the tests are not equivalent, the first will be the more stringent. For it takes account of all sources of variation of which account is taken in the second test other than soil heterogeneity.

MULTIPLE FIELD TRIALS.

Another instance in which two alternative tests of significance arise occurs when we are considering the results of a number of replicated experiments with the same system of treatments carried out at different places and wish to test the significance of the average of some treatment effect over the whole set of experiments. It is only by such series of experiments that results which are reliable and have a wide range of application can be obtained, and the question of the analyses of the results of such a series is, therefore, of considerable importance. Two errors are possible, the first obtained from the interaction of the treatment effect with places, and the second by combining the estimates of error obtained by the analyses of variance at the separate places. Comparison of the two errors by Fisher's z test should always be made, and tells whether the treatment effect differs significantly from place to place. If it does so, in which case the first error is significantly greater than the second, the former, and not the latter, is the appropriate error for testing the significance of the average treatment effect. For the second test takes no account of the variation in treatment effect from place to place. Thus it may, for instance, announce as a significant average response what is really a large response at a few individual places; further it gives no information as to limits within which the average response might be expected to lie if a different set of places were taken. The second test is not in this case either wrong or misleading, but the information which it supplies has ceased to be of much interest.

If it is decided to test the average treatment effects over the whole set of experiments against the interaction with places as error, there is a further point to be borne in mind. Suppose, for example, that there are ten places and that the treatments in each experiment are no manure (0), nitrogen (N), phosphate (P) and the two dressings combined (N × P), so that the three degrees of freedom for treatments split up into three single degrees representing respectively the response to N, that to P and their interaction N × P. The appropriate analysis of variance for examining the treatment responses is

	Degrees of freedom				
Places	9				
			N		1
Treatments	3	←	P		1
			N × P		1
			N	× Places	9
Error 1. Interaction: Treatments × Places	27	←	P	× Places	9
			(N × P)	× Places	9
Error 2. Within places.					

The 27 degrees of freedom for the interaction of treatments with places divide as indicated above into sets of 9 degrees representing the separate interactions of the three treatment effects, N, P and N × P, which have been distinguished. To test the average response to N, however, it is not in general correct to use the whole 27 degrees of freedom as error. For there is no reason to suppose that the variation in response from place to place will be the same for N, P and N × P. The 27 degrees of freedom will therefore not, in general, be homogeneous, and in using the whole set to test individual treatment degrees of freedom we would obtain biased estimates of error. Each treatment effect should on this account be tested against its own interactions with places, so that in the above experiment we would use separate errors for N, P and N × P, each based on 9 degrees of freedom.

SUMMARY.

The study of the effect of rainfall on the yields of wheat from the continuous experiments on Broadbalk, Rothamsted, gave clear evidence of a close relation between the response in yield to rainfall and the manurial treatment of the soil. In later investigations of a similar nature

on barley at Rothamsted and on wheat and barley at Woburn, however, the evidence did not point to a significant effect of rainfall on yields, and on this account little can be said with confidence from these investigations. The present note shows that the relation between seasonal variations in yield and manurial treatment is just as clear at Woburn as on Broadbalk, the difference between the two centres being that similar studies on rainfall effects have had more definite and successful results on Broadbalk. At Woburn, indeed, little progress has been made towards elucidating the particular weather factors whose quantitative influence is important.

For both barley at Woburn and wheat at Rothamsted, which were the cases examined in detail, the grouping of yields according to manurial treatment remained after eliminating the effect of the significant weather factors which were found. This shows that at both centres there are influences, other than rainfall effects of the type examined, whose effect on the seasonal variations in yield is closely associated with manurial treatment.

Some discussion is given of the appropriate test of significance of the difference between two rainfall curves and of a somewhat analogous case which arises in the interpretation of the results of a series of replicated experiments at different centres.

This investigation, and the method of attack, were suggested to me by Mr F. Yates. I am also indebted to Mr Yates for considerable help received during discussion.

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(*Received May 25th*, 1935.)

"SINGLE VALUE" SOIL PROPERTIES: A STUDY OF THE SIGNIFICANCE OF CERTAIN SOIL CONSTANTS.

VIII. RELATIONSHIPS BETWEEN THE STICKY POINT AND THE NATURE OF THE EXCHANGEABLE IONS IN THE SOIL.

By J. R. H. COUTTS.

(*Natal University College, Pietermaritzburg.*)

(With Two Text-figures.)

EARLIER papers in this series (6, 1, 2, 7, 3, 4, 5) have dealt in the main with two principal topics: with considerations of the technique of certain simple physical measurements, and with attempts to elucidate the significance of the results obtained by referring them to the generally accepted notions of the soil from the standpoint of a colloidal complex. E. W. Russell's exhaustive statistical analysis (10) of the writer's data for the Natal soils discussed in Paper II (1) has drawn attention to the complexity of the factors influencing some of the properties measured, and the relative simplicity of others. Russell also brings forward the important new fact that for these soils the base exchange capacity (as measured by Schofield's potassium phosphate method (11)) shows a remarkably high correlation with the sticky point. His correlation coefficient is $r_{BS}=0{\cdot}942$, where B is the base exchange capacity, and S the sticky point.

These Natal soils, and also the majority of the 250 soils for which results were collated by Keen (8), were non-alkaline; for such soils it appears to be definitely established that (within fairly extensive groups of soils) results of practical value can be derived from the moisture equivalent and the xylene equivalent, or from the sticky point and other properties that have been discussed in this series of papers. These statements are, however, valid only for non-alkaline soils; workers on alkali soils have found the sticky point of little value for practical purposes. The difference is, of course, due to the profound alteration in the physical properties of the soil in the presence of sodium as the preponderant exchangeable ion. In the present paper, an account is given of experiments which have been performed in order to examine sticky-point phenomena in homionic soils.

In order to replace the exchangeable ions in the soil by sodium, calcium, or hydrogen, the air-dried (2 mm. sieve) soil was placed in a large glass vessel, and a dilute solution of the appropriate chloride added. The mixture was well stirred and left overnight; the supernatant liquid was then removed from the flocculated material, and a second lot of the chloride added. This second lot was similarly removed the next day, after which the soil was washed repeatedly with distilled water, the liquid being removed through filter candles. The leaching was continued until the washings were free from chlorine and calcium ions, and the samples were then air-dried, broken up with a pestle, and stored until required for use. Although the saturation obtained in this way may not have been quite complete, there is no doubt that the products contained preponderant amounts of the ions specified, and for the purposes of the present work they may be termed sodium soils, calcium soils, or hydrogen soils.

When sticky points were measured in the usual way by kneading the soil with water, it was found that the sticky point for the sodium soil is lower than that for the untreated soil. Examples of this effect are shown in Table I, where the untreated soil is denoted by 0.

Table I. *Sticky points for untreated and sodium soils.*

Soil	0	Na
Rothamsted	28·6	24·4
20/46	30·1	24·3
20/51	40·7	36·0
20/195	41·9	38·7

A detailed study has been made of the sticky-point values obtained when the soils are kneaded with electrolyte solutions instead of water. This work involved the determination (in duplicate in each case) of about 700 sticky points; it included measurements on various soils—both untreated and base saturated—with different electrolytes, each electrolyte being tested over a wide range of concentrations. The chief features of the results are given below.

Table II. *Sticky points for soil 20/46 with various electrolytes.*

log *N*	0	$\bar{1}\cdot7$	$\bar{1}\cdot4$	$\bar{1}\cdot1$	$\bar{2}\cdot5$	$\bar{2}\cdot2$	$\bar{3}\cdot9$	$\bar{3}\cdot3$	$\bar{3}\cdot0$	$\bar{4}\cdot7$
NaCl	30·0	30·0	30·6	31·3	—	31·7	—	—	30·1	—
*Na_2CO_3	28·6	28·4	28·4	29·1	29·6	29·8	—	30·6	—	—
$NaHCO_3$	30·2	30·1	28·8	28·8	28·6	—	30·0	29·0	—	30·1
$CaCl_2$	29·8	30·5	30·9	31·1	—	31·1	31·0	—	—	30·5

* The reduction in sticky point in the case of Na_2CO_3 was observed by Marchand (9) in measuring sticky points of non-alkaline soils with various concentrations of NaCl an Na_2CO_3. The data presented later in this paper suggest that the effect may be due to th dispersive effect of Na_2CO_3.

The sticky points with untreated soils, with calcium soils, and with hydrogen soils show fairly small variations when electrolytes are used instead of water. An example is given in Table II, where the concentrations of the electrolytes are expressed as logarithms of the normality.

With HCl there appears to be a moderate increase of sticky point with decreasing concentration over the range considered; but this case is of less practical importance than the others, and has not been considered in as great detail.

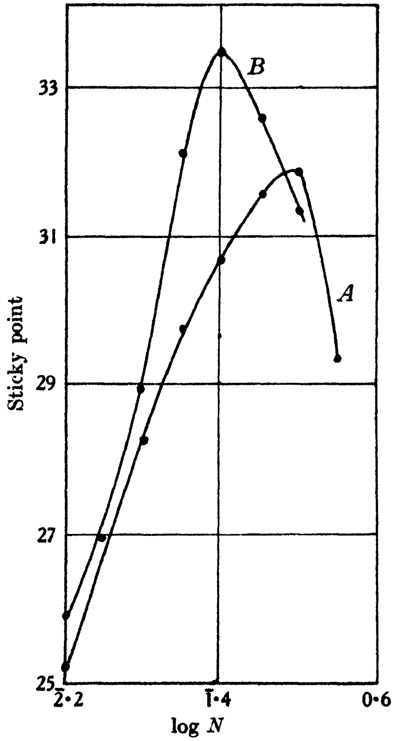

Fig. 1. Sticky points for soil 20/46 Na with varying concentrations of electrolytes.

Curve A NaCl Curve B $CaCl_2$

In the case of sodium soils, the sticky point shows a characteristic and well-marked variation with the electrolytic concentration. The nature of the effect is illustrated in Fig. 1 by two curves with the soil 20/46 Na, using NaCl and $CaCl_2$ as electrolytes. It will be seen that as the concentration decreases each curve rises to a maximum, and then descends steadily; for lower concentrations than those shown, there is in some cases evidence of less pronounced secondary maxima. Curves of similar form were obtained with HCl. With NaOH, the maximum occurred at much higher concentration than for NaCl. A less pronounced maximum was obtained with Na_2CO_3; and with $NaHCO_3$ the sticky point decreased from the greatest available concentration, which is comparatively low.

Thus, while sodiumisation of a soil causes a lowering of the sticky point (as is illustrated by the data in Table I), these further results show that the addition of sodium ions in a solution of fairly high concentration may produce a contrary effect. Since the sodium soil is normally deflocculated, and may become flocculated by the addition of electrolytes, it appeared probable that the observed effects could be accounted for by the state of flocculation of the soil. A series of experiments was performed with the object of testing the validity of this hypothesis.

Suspensions of sodium soils were prepared by shaking together for an hour 2 gm. of the soil with 100 c.c. of electrolytes of various concentrations, and the subsequent course of flocculation observed. To avoid subjective errors, the extent of flocculation was estimated by measuring with a Klett nephelometer the turbidity of the suspension after standing for a few hours. The actual times of standing depended upon the rate of flocculation for the particular series under examination; overnight standing was often found convenient, but shorter periods were sometimes used. *p*H determinations were also made colorimetrically with a Hellige comparator.

There is evidently an enormous difference between the state of affairs in a 2 per cent. suspension and that in a mass of soil at the sticky point, and exact correspondence between the two cannot be expected; but the results show that there is a close relationship between the two effects concerned—sticky point variation and state of flocculation. The extent of the parallelism is indicated by Fig. 2. The data are represented by point-to-point joins, but it may be noted that the decrease in sticky point (by 0·7 per cent.) at the concentration given by $\log N = \bar{1}\cdot 0$ is barely significant, whereas the larger "dip" at a higher concentration ($\log N = \bar{1}\cdot 6$) has been verified by independent redeterminations of the sticky point: the sticky point data plotted in this figure are means of two pairs of duplicates taken at an interval of several days. For concentrations greater than that corresponding to $\log N = \bar{2}\cdot 2$, the similarity between the sticky point and the flocculation curves is as close as can be expected. On the other hand, at lower concentrations there is a second zone of flocculation which is not reflected by the sticky point results.

Correspondences between sticky-point variation and flocculation, to the extent indicated by the results described above, have been found also with other soils; in general, the rise in flocculation values occurs at lower concentrations than the maximum of the sticky-point curve—as might be expected in view of the smaller amount of soil (with its reactive constituents) present. Similar results were obtained also when the sodium soils were kneaded with HCl or with $CaCl_2$, although in such cases the system is more complicated on account of the introduction of the different cation. When the electrolyte was NaOH, however, no correspondence was found. But in this case, at the fairly high concentrations for which flocculation takes place, there must be considerable chemical decomposition of the soil colloids; the solution in the flocculation tubes exhibited a dark brown coloration when free from turbidity.

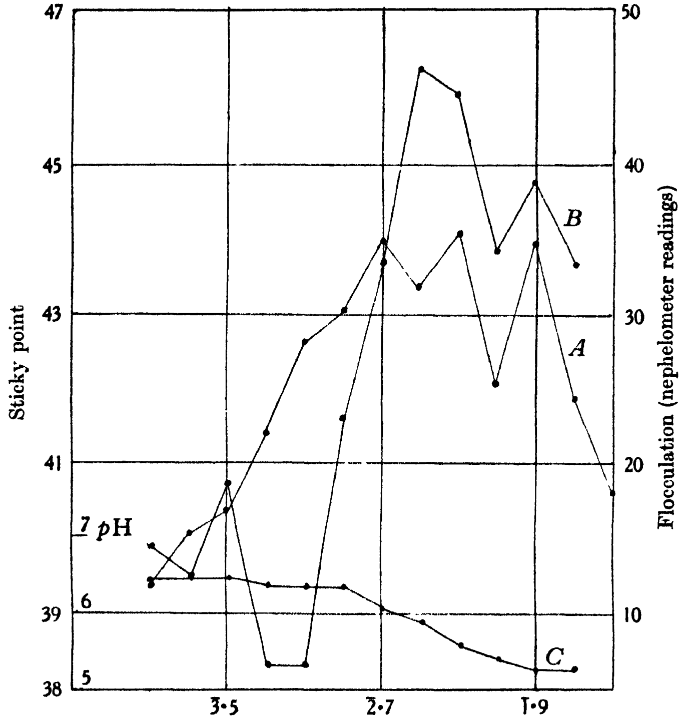

Fig. 2. Sticky point, flocculation, and pH values for soil 20/195 Na with varying concentrations of NaCl.

Curve *A*, sticky point Curve *B*, flocculation Curve *C*, *p*H

A physical interpretation of the differences in sticky points in flocculated and deflocculated soils can be given if we compare the soil structures in the two cases. We may picture the unit of structure in a flocculated soil as a nucleus of soil surrounded by a swarm of more or less loosely attached ions and water molecules, the whole forming a somewhat open sponge-like system. A deflocculated soil is a much finer structure, formed of dispersed soil particles. If this description of the two structures is correct, it is to be expected that more water can be held in the network of the flocculated material than by the dispersed units.

SUMMARY.

The variations in sticky point of homionic soils as determined by kneading with electrolytic solutions have been examined. In the case of sodium soils, a well-marked maximum has been found in the sticky-point/electrolytic concentration curve; and the form of the curve shows parallelism with the extent of flocculation produced by varying electrolytic concentrations.

ACKNOWLEDGMENT.

The preliminary part of this work was done during a period of leave spent at Rothamsted in 1931. The writer gratefully acknowledges the assistance derived from various discussions with the members of the staff of the Soil Physics Department.

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(*Received March* 12*th*, 1935.)

THE COMPOSITION OF CRUDE FIBRE.

By A. G. NORMAN.

(Biochemistry Section, Chemistry Department, Rothamsted Experimental Station, Harpenden.)

INTRODUCTION.

THE determination of crude fibre is one of the oldest analyses made in routine agricultural practice, and one which, if its limitations be fully recognised, is of great value in checking the composition of commercial feeds and similar materials. Because of its adoption for this purpose and its incorporation into the Fertiliser and Feeding Stuffs Acts, this determination seems to have acquired amongst some workers a significance out of all proportion to its real value. While the fraction obtained by this purely empirical method may be taken as an indication of the amount of "bulk" or "roughage" in the material, it cannot be held to bear any definite and regular relationship to any particular plant constituent or group of constituents, or to the crude fibre of any other plant material. Many workers have from time to time drawn attention to the variability of the product obtained, and a number of alternative methods have been proposed, none of which have proved to be in the long run more satisfactory than the original procedure. Very recently, two enzymatic methods have been suggested (Remy (5), Williams and Olmsted (6)) which are theoretically sounder than the drastic treatment with acid and alkali. They are, however, far more tedious and are only suitable for research purposes.

While the shortcomings of the crude-fibre determination have, perhaps, been realised by many agricultural chemists (*vide* C. Crowther (1)), there is a natural reluctance to abandon the method because of the great mass of digestibility data dependent upon it. Recently, however, it has found increasing use in new digestibility studies, and in work on crop composition as affected by manurial or other conditions, for which purposes it is wholly unsuited. Results obtained are apt to be misleading, since the composition of the "crude fibre" fraction is not constant. The time is approaching, or has already been reached, when experiments in animal nutrition concerned with the availability of plant materials should be conducted in terms of the actual plant constituents rather than in terms of empirical figures such as crude fibre. A multitude of digestibility experiments may have to be repeated, but the gain in precision that would be achieved would make this worth while. The crude-fibre determination will remain for the ordinary routine purposes for which it is well suited, if the interpretation placed upon it be revised and restricted somewhat. In this paper, results are presented which demonstrate the nature of the shortcomings and magnitude of the errors in this determination.

EXPERIMENTAL.

Detailed analyses were made on a wide range of agricultural materials, on the crude-fibre fraction from these materials, and also on the residue at the end of the 1·25 per cent. acid treatment, before the final 1·25 per cent. NaOH extraction. In this way it was possible to decide what exactly the crude-fibre fraction is composed of in terms of plant constituents, what proportion of the amount present in the original material these represent, and, finally, what is the effect of each step in the process of isolation of this fraction.

The materials employed were mature bean straw, mature oat straw, green wheat plants six weeks before harvest, mature hay, three samples of vetches cut at different periods, young green plants of maize and of kale, sugar-beet pulp, and bran.

The analyses carried out were:

(1) *Cellulose* by the method of Norman and Jenkins (2).

(2) *Furfuraldehyde* yield by the usual phloroglucide procedure. The difference between total furfuraldehyde yield and furfuraldehyde from the natural cellulose fraction represents furfuraldehyde due to the polyuronides or encrusting hemicelluloses, and, in the absence of any satisfactory method for their estimation directly, may be taken as a measure of this group.

(3) *Lignin* after pre-hydrolysis with 5 per cent. H_2SO_4 as described by Norman and Jenkins (3).

(4) *Protein*, N × 6·25.

(1) *Composition of the crude-fibre fraction.*

The crude-fibre fraction of a wide range of materials was prepared and analysed by the methods just mentioned. It was found to consist almost exclusively of cellulose and lignin, the figures for which are given in Table I. On an average, about 97 per cent. of the fraction could be accounted for in terms of these two constituents, a result which indicates that the procedure is effective in excluding other constituents. Attention is drawn, however, to the very variable lignin content of the crude-fibre fractions, and to the fact that a crude fibre high in lignin was not necessarily obtained from the more highly lignified materials.

If, however, these analyses are expressed on an equivalent basis, and compared with the analysis of the original material as in Table II, it

Table I. *Amount and composition of crude-fibre fractions.*

		Composition of crude fibre	
Material	Crude fibre, % of dry matter	Cellulose %	Lignin %
Bean straw	45·8	76·8	18·6
Oat straw	35·2	92·0	6·8
Wheat plants	32·6	92·4	6·4
Hay	31·5	85·7	10·9
Vetches 3	31·1	74·3	20·2
Vetches 2	30·0	79·3	19·1
Vetches 1	22·9	77·0	17·1
Maize plants	22·6	96·5	3·5
Kale plants	17·3	76·2	18·1
Sugar-beet pulp	14·5	87·9	10·6
Bran	11·3	93·0	2·1

Table II. *Comparison of the composition of crude fibre with that of the original material. All results expressed as percentage of original oven-dry material.*

Material	Crude fibre	Natural cellulose	Xylan in cellulose	Lignin	Furfuraldehyde from hemicellulose	Protein
Bean straw	45·8	41·9	9·0	12·7	5·1	4·3
C.F.*	—	35·1	5·6	8·5	0·7	0·4
Oat straw	35·2	43·7	12·1	11·4	7·6	3·4
C.F.	—	32·4	3·6	2·4	1·2	0·2
Wheat plants	32·6	43·0	9·7	13·1	7·6	8·2
C.F.	—	30·2	4·4	2·1	0·3	0·2
Hay	31·5	37·7	9·6	9·8	5·9	9·1
C.F.	—	27·0	3·9	3·5	0·3	0·3
Vetches 3	31·1	31·8	6·3	14·3	7·5	15·8
C.F.	—	23·1	3·7	6·3	0·9	0·5
Vetches 2	30·0	27·2	4·1	12·9	5·5	21·0
C.F.	—	23·8	3·1	5·7	0·9	0·8
Vetches 1	22·9	23·7	3·0	11·2	3·8	30·5
C.F.	—	17·7	2·0	3·9	0·5	1·1
Maize plants	22·6	36·6	9·2	8·0	5·3	15·3
C.F.	—	22·0	2·8	0·8	0·4	0·1
Kale plants	17·3	18·5	2·9	5·7	5·8	26·8
C.F.	—	13·2	2·0	3·1	0·3	0·2
Sugar-beet pulp	14·5	18·9	1·8	6·9	11·1	†
C.F.	—	12·8	0·8	1·5	0·0	†
Bran	11·3	26·3	11·1	6·0	4·4	†
C.F.	—	10·5	0·5	0·2	0·1	†

* C.F. = crude fibre. † Not determined.

will be seen that the cellulose and lignin of the crude-fibre fraction does not by any means represent all the cellulose and lignin initially present. The extent of the recovery in each case is given in Table III, in which the cellulose and lignin of the crude fibre are calculated as percentages of those present in the original material.

Table III. *Recoveries of cellulose and lignin in the crude fibre. Each expressed as percentage of that present in original material.*

Material	Cellulose	Lignin
Bean straw	84	67
Oat straw	77	21
Wheat plants	71	14
Hay	72	35
Vetches 3	73	43
Vetches 2	88	44
Vetches 1	75	35
Maize plants	60	10
Kale plants	72	55
Sugar-beet pulp	68	22
Bran	40	4

While the cellulose, with one exception, did not show any very wide variation, and represented a recovery of 60–80 per cent., the lignin showed a much greater variability, from 4 to 67 per cent. of that present in the original material going through into the crude fibre. These analyses provide sufficient support for the view expressed previously, that the crude-fibre fraction does not represent any constant fraction of the plant constituents, and that the crude fibre from one material is not necessarily comparable in composition with this fraction from another.

(2) *Composition of the fraction obtained by acid treatment only.*

In order to discover, if possible, the reason for the failure of the crude-fibre method to give a more constant fraction, similar detailed analyses were carried out on the same materials at the end of the treatment for 30 min. with 1·25 per cent. acid. In this way, a decision can be reached as to whether the acid or the subsequent alkaline treatment introduces these defects. Table IV presents the analyses of cellulose and lignin in the acid-extracted residues. The lignin content of these residues was uniformly much higher than in the crude-fibre fractions, and the cellulose lower. The total of these two, however, was far from 100 per cent., and the detailed analyses given in Table V show that the acid residue still contained hemicelluloses, and protein also. The subsequent alkali treatment in the complete crude-fibre procedure is apparently successful in removing virtually all this remaining hemicellulose and

Table IV. *Amount and composition of acid-extracted material.*

Material	Acid-extracted residue, % of dry matter	Composition of acid-extracted residue	
		Cellulose % of A.E.	Lignin % of A.E.
Bean straw	60·7	64·7	19·6
Oat straw	56·2	67·9	15·5
Wheat plants	58·0	62·1	18·8
Hay	54·1	64·9	21·1
Vetches 3	51·8	55·4	23·7
Vetches 2	48·0	53·9	24·5
Vetches 1	45·5	48·9	22·0
Maize plants	45·5	57·0	19·2
Kale plants	29·8	64·0	17·3
Sugar-beet pulp	25·6	71·0	22·7
Bran	18·5	68·1	25·2

A.E. = acid-extracted residue.

Table V. *Comparison of the composition of acid-extracted residue with that of the original material. All results expressed as percentages of the original oven-dry material.*

Material	Acid-extracted residue	Natural cellulose	Xylan in cellulose	Lignin*	Furfuraldehyde from hemicellulose	Protein
Bean straw	60·7	41·9	9·0	12·7	5·1	4·3
A.E.	—	39·3	6·4	12·2	2·6	1·8
Oat straw	56·2	43·7	12·1	11·4	7·6	3·4
A.E.	—	38·0	5·9	10·5	3·0	1·3
Wheat plant	58·0	43·0	9·7	13·1	7·6	8·2
A.E.	—	36·0	7·3	10·9	2·6	2·2
Hay	54·1	37·7	9·6	9·8	5·9	9·1
A.E.	—	35·2	5·7	9·3	3·0	4·7
Vetches 3	51·8	31·8	6·3	14·3	7·5	15·8
A.E.	—	28·7	4·4	12·3	2·9	6·4
Vetches 2	48·0	27·2	4·1	12·9	5·5	21·0
A.E.	—	25·9	3·3	10·8	4·6	8·6
Vetches 1	45·5	23·7	3·0	11·2	3·8	30·5
A.E.	—	22·2	2·0	10·0	1·5	12·5
Maize plants	45·5	36·6	9·2	8·0	5·3	15·3
A.E.	—	26·0	4·5	8·7	1·7	7·4
Kale plants	29·8	18·5	2·9	5·7	5·8	26·8
A.E.	—	18·1	2·3	5·2	1·5	3·4
Sugar-beet pulp	25·6	18·9	1·8	6·9	11·1	†
A.E.	—	12·8	0·8	1·5	0·0	†
Bran	18·5	26·3	11·1	6·0	4·4	†
A.E.	—	12·6	1·0	5·4	0·0	†

* For reasons which have been fully discussed elsewhere (Norman and Jenkins (4)) the lignin determination on materials high in nitrogen is not so accurate as in those low in nitrogen. Certain apparent discrepancies occur in these and later figures due to this cause.

† Not determined.

protein. Comparison of Tables II and V shows that the action of the alkali goes further, producing an extensive removal of lignin, and also an additional attack on the natural cellulose, largely, though not exclusively, by removal of xylan in cellulose. Table VI gives the recoveries of cellulose and lignin in the acid-extracted residues for comparison with Table III. The acid treatment produced only a limited attack on the cellulose, and a slight effect on the lignin. Indeed, on theoretical grounds lignin might be held to be unaffected by such dilute acid, and the differences observed ascribed to the disturbance caused by protein in the lignin determination. This, however, though believed to be the case is not yet proved, and is being investigated in another connection.

Table VI. *Recoveries of cellulose and lignin in the acid-extracted residues. Each expressed as percentage of that present in the original material.*

Material	Cellulose	Lignin
Bean straw	94	96
Oat straw	87	92
Wheat plants	84	83
Hay	94	95
Vetches 3	91	86
Vetches 2	96	84
Vetches 1	94	90
Maize plants	71	100 +
Kale plants	98	91
Sugar-beet pulp	96	99
Bran	48	91

The information given by these analyses makes it possible to understand the action of the reagents used in each stage of the isolation of crude fibre.

Plant constituents	Effect of 1·25 % acid. 30 min.	Effect of 1·25 % alkali. 30 min. following acid extraction
Natural cellulose:		
True cellulose	Very slight	Slight
Cellulosan	Slight hydrolysis	Partial extraction
Encrusting hemicelluloses, or polyuronides	Extensive hydrolysis and extraction	Complete extraction
Starch	Hydrolysis and extraction	—
Lignin	Slight, if any	Extensive extraction (rather variable)
Protein	Partial extraction	Almost complete extraction

From the literature, it is clear that the interpretation put upon the crude-fibre fraction varies amongst different workers. Some appear to hold that it represents only the cellulose. If this were to be the case, the present method would be unsatisfactory in that the cellulose is partially attacked but by no means completely freed from lignin. Other workers are of the opinion that it represents all the cellulose plus all the lignin. in other words the structural framework of the material. Woodman and Stewart (8) apparently support this view, for in discussing the crude-fibre determination, they state that: "It is exceedingly probable that this treatment does not actually dissolve out more than insignificant amounts of lignocellulose." As has been seen, the lignin does, however, suffer extensive losses, to a degree which varies rather widely with the material, and in addition the cellulose is partially attacked. The conclusion appears inevitable that the crude-fibre determination as carried out at present is misleading and unsatisfactory for research purposes, and can be taken only as an approximate indication of bulk or roughage. Even so, the estimate made would be on the low side, for the reasons just given.

This variability in the composition of the crude-fibre fraction has several important implications, and is opposed to the assumption apparently made by some workers in animal nutrition. In the first place, it cannot be assumed that the changes which take place in lignification will be paralleled by changes in the "crude fibre" as isolated. This fact explains, for example, certain inconsistencies found by Woodman and Stewart (8) in their investigation of the digestibility of the fibre from some typical feeding stuffs. By the use of a culture of an active cellulose-decomposing organism they endeavoured to assess digestibility, and while this ingenious method was partially successful, the differences observed in extent of fermentation of the fibre were not nearly so great as was expected from the known digestibility coefficients of the materials from which the fibre samples were prepared. The loss of lignin during the isolation of the fibre samples had the effect of smoothing out the differences between the materials. This was particularly evident in the case of samples of perennial rye-grass taken at seven different stages of growth. The "fermentation coefficients" as determined by means of the cellulose organism fell only from about 85 per cent. in the case of the youngest green leaf to 73 per cent. for the fully mature grass with the seeds formed, while it is well known that digestibility is reduced by lignification and maturity in a much greater proportion. They explained these differences by stating that the digestibility of a crop is not necessarily influenced by the amount of "lignocellulose" present but is primarily related to the manner of its deposition, and the intimacy of its association in the cell wall. An alternative and more reasonable explanation arises out of the work described in this paper, and would appear to be that the crude fibre as isolated is not truly representative of the material from which it comes.

Perhaps a more serious implication of this work is that it calls into question the basis upon which digestibility coefficients of the fibre of feeding stuffs are determined and calculated. It is always assumed that the residual fibre in the faeces is directly comparable with that from the original material fed to the animal. This seems to be a doubtful assumption, and one which should be submitted to a careful examination. The changes which take place during its passage through the digestive tract, the partial utilisation of cellulose, the extensive removal of starch, hemicelluloses and proteins, completely changes the nature of the material. Accordingly, when a crude-fibre determination is carried out on the faeces, it is hardly likely that the percentages of the residual cellulose and lignin that go through into the crude-fibre fraction will be the same as in the material originally fed. A detailed series of analyses on the food material and the faeces, and the crude fibre from each would settle this important point.

(3) *The requirements of any method of assessing fibre.*

If it be accepted that the less digestible part of plant tissues is represented by the cellulose and lignin, then any empirical "fibre" method should leave a residue consisting of these two, as far as possible unchanged and unattacked. Not all workers, perhaps, will accept this definition of fibre. For example, Williams and Olmsted (6) in the investigation of human faeces define "non-digestible residue as those vegetable materials not attacked by digestive enzymes in the mammalian gut, and consisting of lignin, cellulose, and non-water-soluble hemicelluloses". The distinction is, however, between indigestible residue and fibre.

Lignin as occurring in plants is in close association with cellulose in the cell wall, though not combined with it. There is no evidence for the existence of a "lignocellulose" other than as a mechanical or physical association. Herbivorous animals are capable of digesting cellulose completely, not directly, but by the agency of cellulose-decomposing bacteria in the digestive tract. In the young plant the cell walls are thin and more or less unlignified. Digestion coefficients of the fibre of young pasture herbage have been found to be as high as 89 per cent. (Woodman (7)). Lignin, on the other hand, is, by the concensus of opinion, held to be either entirely indigestible or almost indigestible, and because of its distribution hinders the attack on the cellulose itself. The onset of lignification is, therefore, accompanied by a steady diminution in the digestibility of the cellulose in the crop. The consequences of lignification, however, extend further than that, and unless the material is supplied to the animal in a very finely divided condition, the utilisation of proteins and available carbohydrates is hindered, as this largely depends on the extent to which the cell wall is attacked. As a result, it is probably true to say that the digestibility of any normal plant material is very roughly proportional to the degree of lignification, or its lignin content. Therefore, an essential requisite of any method, either empirical or scientific, for evaluating the more resistant cell-wall material is that a fraction should be given which includes all the lignin, and not just a small and variable part, as in the present "crude-fibre" determination. In order to achieve this, any sort of alkaline treatment must be dispensed with, since the experiments presented here show that the alkaline extraction was accompanied by grave losses of lignin. Ideally, a knowledge of the actual lignin content of the material itself or of its fibre fraction is desirable, and presents no special difficulties. On mature materials the lignin determination may be taken as accurate, but on green or nitrogenous materials errors are introduced by the presence of protein, and the apparent lignin content is increased above the true value (Norman and Jenkins (4)).

(4) *Acid hydrolysis as a possible alternative empirical method.*

In digestibility work a knowledge of the actual composition of the material would make for increased precision. Under certain circumstances, sufficient information might be obtained from an approximate figure for cellulose + lignin, together with the actual lignin content. While the 1·25 per cent. acid treatment given in the present crude-fibre method does not bring about any very serious attack on either the cellulose or lignin, other constituents are not sufficiently removed, and, as seen in Table IV, the cellulose and lignin taken together amount usually to only about four-fifths of the residue. It seemed possible, however, that by increasing the acid concentration, or by lengthening the time of boiling, a more extensive hydrolysis might be effected while still retaining all or practically all of the lignin and without having too drastic an effect on the cellulosan. Hydrolysis residues were prepared from oat straw, bean straw and mature hay by boiling with 3 per cent. acid for 30 min. Table VII gives the chief analyses of these samples calculated both on the residues and as percentages of the amount present in the original material. The sum of cellulose + lignin was well below 100, indicating that other constituents still remained. In addition, there was a partial attack on the cellulose, inevitable in any treatment either acid or alkaline. By the employment of a higher concentration of acid and longer time of boiling—5 per cent. H_2SO_4 for 1 hour—a more extensive hydrolysis was achieved. The cellulose was attacked rather vigorously, but, at the same time, encrusting materials were more effectively removed. Cellulose and lignin taken together accounted for about 90 per cent. of the residue for these same materials, as shown in Table VIII.

Table VII. *Hydrolysis of materials by boiling 3 per cent.* H_2SO_4 *for 30 min.*

Material	Residue, % of dry matter	Expressed as % of residue		Expressed as % of that in original material	
		Cellulose	Lignin	Cellulose	Lignin
Bean straw	61·4	61·1	20·0	89	97
Oat straw	53·4	66·8	19·8	82	95
Hay	51·7	64·5	20·0	88	100 +

Table VIII. *Hydrolysis of materials by boiling 5 per cent.* H_2SO_4 *for 1 hour.*

Material	Residue, % of dry matter	Expressed as % of residue		Expressed as % of that in original material	
		Cellulose	Lignin	Cellulose	Lignin
Bean straw	50·3	64·5	25·1	78	100 +
Oat straw	50·0	66·1	24·2	76	99
Hay	41·9	62·8	26·2	70	100 +

As a purely empirical method on such materials, this might be considered a sufficiently close approximation, as the loss in cellulose is roughly balanced by the non-cellulosic material remaining. An estimate of "fibre" made on this basis would be rather on the low side.

However, this is not a procedure which could be generally applied without some modification, because in the case of younger materials high in nitrogen such an acid treatment is not effective in the removal of protein. This may be allowed for by a nitrogen determination on the residue. The protein-free residue obtained is rather lower in all cases than the total cellulose + lignin present originally, and the acid treatment might have to be either a little briefer, or carried out with a lower concentration of acid on this type of material to obtain a closer agreement. Analyses made on some materials high in protein are given in Table IX.

It is not necessarily to be expected that one concentration of acid will be found to be uniformly suitable for all classes of material. This phase of the work will receive further attention.

Table IX. *Hydrolysis of nitrogenous materials by boiling 5 per cent.* H_2SO_4 *for 1 hour.*

Material	Residue, % of dry matter	Expressed as % of residue			Expressed as % of that in original material		
		Cellulose	Lignin	Protein	Cellulose	Lignin	Protein
Vetches 1	37·6	49·4	32·7	22·8	79	100+	29
Vetches 2	40·4	57·1	33·9	14·4	85	100+	28
Vetches 3	43·3	59·4	32·2	11·6	78	97	32
Wheat plants	49·4	68·1	26·2	4·5	78	91	55

If the figures in Tables VIII and IX are given in the way that the suggested empirical determination would be used, the estimate of cellulose would be low in all cases, partly due to the attack on cellulosan and partly because of the high apparent lignin content given by nitrogenous materials. These results are given in Table X. A closer approximation might be obtained by a different acid concentration, but the precise strength would have to be determined for each material under investigation.

Table X. *Estimate of cellulose content made by acid hydrolysis and determination of lignin and protein. All results expressed as % of original dry material.*

Material	Residue	Lignin	Protein	Cellulose (by difference)	Cellulose actual
Bean straw	50·3	12·6	*	37·7	41·9
Oat straw	50·0	12·2	*	37·8	43·7
Hay	41·9	11·0	*	30·9	37·7
Vetches 1	37·6	11·0	8·6	18·0	23·7
Vetches 2	40·4	12·3	5·8	22·3	27·2
Vetches 3	43·3	12·5	5·0	25·8	31·8
Wheat plants	49·4	11·6	2·2	35·6	43·0

* Not determined.

SUMMARY.

The crude-fibre determination is misleading, because the fraction obtained does not bear any definite relationship to the structural constituents of the material. The cellulose is partially attacked, and lignin extensively removed. Considerable variation is found in the lignin content of crude-fibre fractions. Highly lignified materials do not necessarily yield a crude fibre high in lignin.

Since the presence of lignin exercises a direct effect on the digestibility of the material, any empirical method should include all the lignin, and alkaline treatment must be avoided. Acid hydrolysis may be a possible alternative method, if a correction for protein be made in the case of materials high in nitrogen, and a determination of the lignin content made.

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(*Received June 5th*, 1935.)

NOTE ON THE EFFECT OF "CONDITION" ON THE COLOUR OF BODY FAT IN RABBITS.

BY R. HIRZEL.

*(Stellenbosch University, South Africa, and
School of Agriculture, Cambridge.)*

INTRODUCTION.

FAT colour is of importance in beef produced for English markets because of discrimination against deep yellow fat. The factors at present known to affect colour are genetic (1, 4) and those relating to food supply (2, 3), but not much is yet known of the various circumstances or natural conditions under which the colour will vary in shade. It was the purpose of this investigation to determine some of these. Rabbits were selected for the preliminary experiments, as, although it is questionable whether the colouring matter in the fat is quite the same as in cattle (3, 5), it is very probably affected by the same conditions. Experiments could be done quicker and cheaper in rabbits than in cattle on which the results could be tested after the pioneer work in the rabbit was completed.

EXPERIMENTAL.

An inbred strain of rabbits, in which the factors for yellow fat have been fixed, formed the material for this investigation. During post-mortem examinations it was observed that in lean animals the body fat was of an unusually dark yellow colour; data of a preliminary nature were collected to ascertain the extent of the difference in fat colour

Table I. *Fat rabbits.*

Rabbit No.	Sex	Age months	Weight at killing gm.	Colour of kidney fat grade	Condition
1	♂	43	3865	9	Very good
2	♂	26	3410	8·5	Good
3	♂	10½	2745	9	Good
4	♀	40	4593	9	Very good
5	♀	25½	4181	9	Good
6	♀	12	3265	8	Good
7	♀	11½	3503	9	Good
8	♀	10½	3434	8·5	Fair
9	♀	8½	3360	8	Good
10	♀	6	2994	6	Good
11	♀	8	3270	4	Fair
12	♀	6½	3565	4	Good

between lean and fat animals. In measuring the colour a scale developed by Hammond, consisting of a series of "blads" ranging from very light yellow to dark yellow and numbered from 0 (lightest) to 13 (darkest), was used.

Fat-colour readings were taken of fat from various parts of the body. The shoulder fat occasionally appeared to be somewhat lighter than other fats, but in the majority of cases it was the same colour as that of the kidney. Kidney fat was found to be the most reliable source; it has a small percentage of connective tissue, forms a solid mass, and is present in lean animals when fat from most of the other body parts is absorbed. Data from a series of rabbits which had grown normally and had never had any serious set-back are presented in Table I. The majority were killed in good condition. The light fat of No. 10 is due to the fact that its growth was made during the winter months when green food containing xanthophyll, the chief pigment in rabbit fat (5), was absent from the diet. Although No. 12 was fed on a large percentage of carrots the colour of the fat was not affected, as the colouring matter in carrots is chiefly carotin. No. 11 deposited its fat at a time when green food was absent from the ration.

Because these young rabbits were fed differently from the older ones it is not possible to decide from these data whether there is any connection between age and amount of pigment deposited in the fat. No sex differences are apparent. Table I indicates that in rabbits fattened in the normal way the colour averages 8–9 by the scale.

Table II. *Lean rabbits (bucks).*

Rabbit No.	Age at highest weight months	Age when killed months	Highest weight gm.	Weight when killed gm.	Drop in weight gm.	Percentage drop in weight	Colour of kidney fat grade	Condition
1	31	37	3295	2625	670	20	13	Very lean
2	1	1¼	838	670	168	20	12	Very lean
3	13	15	3570	3035	535	15	11	Lean
4	23	26	3175	2890	285	9	10	Fair
5	19	23	3385	3135	250	7	11	Fair–lean

The result of loss in condition can be seen in Table II. The fat becomes a dark yellow colour and in extreme cases is almost orange. The extent to which this orange tint may be caused by contraction into a small area of capillaries containing blood is uncertain, but microscopic examination shows that the yellow fat cells are definitely darker in colour than the normal. Compared with the fat of normal rabbits, that of these lean ones is 2–4 shades yellower.

Table III. *Experimental rabbits* (*bucks*).

Rabbit No.	Age when killed months	Starved weight at operation gm.	Weight when killed gm.	Drop in weight gm.	Percentage drop in weight	Colour of fat at operation (shoulder) grade	Colour of fat at killing (kidney) grade	Condition
1	9½	2731	1890	841	31	6	13	Very lean
2	21½	3422	2637	785	23	7·5	9	Fair
3	15½	3275	2560	715	22	7	8	Very good
4	18	3540	2820	720	20	8	10	Good
5	19¾	3080	2480	600	19	8	13	Very lean
6	10¼	3225	2930	295	11	4	6	Good
7	21	3437	3090	347	10	8·5	9	Fair

Experiments on fat-colour changes in individual rabbits substantiate the above results. Buck rabbits were fattened to a high condition and samples of fat from the shoulder strap removed for colour readings. The animals were then placed on a submaintenance ration (on which they rapidly lost weight) and were killed at various stages when further readings were taken, this time from the kidney fat. Unless the rabbit is in good condition its shoulder fat frequently appears to be somewhat lighter in colour than the kidney fat on account of the large amount of connective tissue present. On rendering, however, that is heating the fatty tissue to extract the fat, it is seen that the shades of shoulder and kidney fat are identical in colour. Readings from the shoulder fat (taken when rabbits were in good condition) are consequently compared with readings from the kidney fat (taken at time of killing when rabbits were leaner). The results are shown in Table III. In most cases the fat had deepened noticeably in colour, particularly when animals had become very lean. The reason for a fairly low fat colour in Nos. 1 and 6 is that they were fattened on a diet containing a low percentage of xanthophyll.

DISCUSSION.

The tentative conclusion suggested by these results is that in fattening a proportion of the ingested pigment is laid down in the fat, but that when such fat is used for body maintenance the pigment is not wholly reabsorbed but concentrated in the fatty tissue, thereby deepening the colour.

The results given here are only preliminary experiments and are now reported because owing to the author's return to South Africa the work had to be discontinued. It requires to be extended under conditions where the fat colour can be tested more accurately. Nevertheless, the results suggest an explanation for the fact that the fat colour in old cows and steers which have undergone seasonal fluctuations in condition is usually a darker tint than in young heifers and steers which have fattened steadily, and that animals once fat and killed in poor condition are usually darker in colour than fat ones.

ACKNOWLEDGMENTS.

I am greatly indebted to Dr John Hammond, Animal Nutrition Research Institute, School of Agriculture, Cambridge, for providing the material (rabbits of his E strain) from which these data have been collected, and to Miss E. O. Whetham and Dr A. Walton who undertook the operations.

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(*Received June* 14*th*, 1935.)

THE ESTIMATION OF THE EFFICIENCY OF SAMPLING, WITH SPECIAL REFERENCE TO SAMPLING FOR YIELD IN CEREAL EXPERIMENTS.

By F. YATES, M.A. and I. ZACOPANAY, B.A.

(*Statistical Department, Rothamsted Experimental Station, Harpenden.*)

(With Two Text-figures.)

I. Introduction.

The estimation of the yields of the individual plots of replicated experiments on cereals by sampling methods has been practised since the year 1929 at Rothamsted and its associated outside centres. A report on the results obtained in the first four experiments harvested by this method, three of which were also harvested in full, was published by Clapham in 1931 (2, 3). Since then the method laid down by Clapham has been followed with little alteration, except for a change of form of sampling unit, introduced as a result of Kalamkar's work of 1932 (4). There is thus a considerable body of results available, in all of which the sampling errors can be estimated, and the efficiency of the sampling process as a method of harvesting assessed.

Clapham came to the conclusion that with the amount of sampling practised by him (about 6 per cent. of each plot excluding edges) there would be "little loss of information". His own results, however, scarcely bear out this contention, since the actual estimates of the percentage losses of information (calculated by the method given later) in the grain yields of the two barley experiments and the oats experiment are 31·0, 49·8 and 19·8 respectively. The wheat experiment, the only one with negligible loss of information, Clapham dismissed from consideration because of its excessive error. It is true that the comparison of the experimental errors of the sampling results and those of the full harvestings given by Clapham may have created the impression that the losses of information were smaller. This, however, is entirely due to one very low sampling experimental error, which was unfortunately computed wrongly. When this is set right there is no evidence so far as the grain is concerned that harvesting the whole of the experiment by sampling methods would differ materially from full harvesting; consequently the losses given above may be considered reliable.

The present paper contains an investigation of the actual efficiency of the sampling processes adopted in the Rothamsted experiments, as revealed by the sampling and experimental errors. Opportunity is taken to review the theory of sampling errors, and their estimation. It will be shown that with a given relation between experimental and sampling variation and between the work involved in sampling and in the rest of the experiment there is an optimal percentage of sampling. It will also be shown that in many cases there is little to be gained by pushing the sampling beyond certain fairly well-defined limits, even when the work involved in sampling is small.

This discussion is of importance not only in considering the application of sampling to the estimation of yield, but also in all sampling processes performed on replicated experiments. The estimation of plot yields by sampling is an application of only minor importance, since it is always possible, and often simpler, to harvest the whole of each plot; but in many other cases sampling is a vital necessity, and results can be obtained by its aid which could otherwise only be obtained with excessive labour, or not at all. The determination of the necessary amount of sampling in such cases, and the balance between the sampling and the size of the experiment, is a problem which continuously confronts the experimenter. In particular the individual observation of every plant, of every tree, or of every animal in an experiment, may often prove to be unnecessary when consideration is given to the amount of information obtained.

The paper also contains suggestions for certain modifications of the present sampling procedure for cereal crops. The most important of these (already employed by some workers) is the use of sampling to determine the proportion of grain to total produce on each plot, the yield of total produce being determined by full harvesting. It will be shown that this method is capable of greatly reducing the sampling errors with considerably smaller samples than are at present taken, and it seems likely that the method will prove a useful alternative to the present method. The details of an efficient field technique remain to be worked out and tested in practice before it is possible to make a definite assessment of the relative advantages of the two methods.

II. THE INTERPRETATION OF THE ANALYSIS OF VARIANCE IN SAMPLING PROBLEMS.

Before proceeding to any discussion of the efficiency of sampling it will be worth considering the exact interpretation of the analysis of variance which we shall employ to evaluate the sampling and experimental errors.

The terminology laid down by Wishart and Clapham (5) will be followed with the exception of the word *unit*, which they use to denote "the ultimate parts of a sample—for cereals, short lengths of drill, or small areas; for roots and potatoes, individual plants, etc." Since there is nothing ultimate about a short length of drill row, which may always be subdivided into shorter lengths, and since, moreover, this use of the word unit is likely to be confused with the *sampling unit*, which they define as "those parts of the sample which are located independently and at random within the area to be sampled", it has appeared to us preferable to refer explicitly to any parts of the sampling unit which we wish to consider. As with the above writers the word *sample* will be taken to denote an aggregate of sampling units, such as those from a given plot in an experiment.

We will first consider the simple case in which there are l plots (all treated alike) and each plot is subdivided into h equal parts, of which a random selection of number k are harvested and separately weighed. There are thus h possible sampling units per plot of which k are taken.

In all probability neighbouring sampling units will be more alike than those far apart, but since we are not prepared to consider the position of each individual unit we must assume that all units in the same plot have the same variance and the same correlation with one another; this is quite legitimate, since the actual units chosen are selected at random from the h possible sampling units.

We may proceed to perform an analysis of variance within and between plots. It will be best to work in units of a single sampling unit. The analysis can be represented symbolically as follows:

	Degrees of freedom	Sum of squares	Mean square
Between plots	$l-1$	$(l-1)A$	A
Within plots	$l(k-1)$	$l(k-1)B$	B
Total	$lk-1$	—	—

Instead of considering the total sampling yield of each plot it will be more convenient in what follows to consider the mean yield per sampling unit of each plot. The between-plot sum of squares $(l-1)A$ is merely

k times the sum of the squares of the deviations of these plot means, while the within-plot sum of squares $l(k-1)B$ is made up of the total of all the sums of squares of deviations of the yields of the individual sampling units from their own plot means. Thus A/k is the estimate of the variance of the mean yields per sampling unit of the plots amongst themselves, and B is the estimate of the variance of an individual sampling unit, excluding any variation which is common to all the units of a plot.

If no competition exists the yields of the samples of any one plot may be regarded as made up of the sum of a part which varies independently from sample to sample, and a part which is constant and affects all samples of the plot equally, but varies from plot to plot. The first part, for example, might be mainly due to broad soil irregularities and the second to local soil irregularities and plant variation.

The first part may be taken as varying about zero with variance V_S, but the mean for any one plot will not be exactly zero, the variance of this mean (for all h sampling units) being V_S/h. The second part (variance V_P), being the same for all sampling units of the plot, will appear unchanged in the plot mean, and consequently the total variance of the plot mean will be

$$V_P + V_S/h,$$

or if only k sampling units are taken,

$$V_P + V_S/k.$$

If competition between samples exists a further term must be added to the other two. The variation of this term may be taken to be such that the sum over all the sampling units of the plot is zero, so that if for instance one unit yields high this is exactly compensated for by some or all of the others yielding low. If $c_1, c_2, \ldots, c_h$ are independently distributed with variance V_C and $\bar{c}$ is their mean,

$$c_1-\bar{c},\ c_2-\bar{c},\ \ldots,\ c_h-\bar{c},$$

will fulfil this condition.

Since the mean of the first k of these quantities is equal to

$$\frac{h-k}{hk}(c_1+c_2+\ \ldots\ +c_k)-\frac{1}{h}(c_{k+1}+\ \ldots\ +c_h),$$

the variance of this mean is

$$\left[k\left(\frac{h-k}{hk}\right)^2+(h-k)\left(\frac{1}{h}\right)^2\right]V_C=\frac{(h-k)}{hk}V_C.$$

In calculating the variance within plots we are concerned with the differences between pairs of sampling units, $\bar{c}$ cancels out, and the resultant variance is therefore

$$V_S + V_C.$$

The total variance of the plot means (of k sampling units) will be

$$V_P + \frac{1}{k} V_S + \frac{h-k}{hk} V_C = \left(V_P - \frac{1}{h} V_C\right) + \frac{1}{k}(V_S + V_C),$$

and if the whole plot had been sampled it would be

$$V_P + \frac{1}{h} V_S = \left(V_P - \frac{1}{h} V_C\right) + \frac{1}{h}(V_S + V_C).$$

Thus the effect of competition can be deduced from the case where the variation was represented by two independent parts by replacing V_S by $V_S + V_C$ and V_P by $V_P - (1/h) V_C$. In what follows we will denote these quantities by V_S' and V_P'.

In the limiting case where the whole of the variation is due to competition the variance between plots would be zero if the whole plot were sampled, and

$$\left(\frac{1}{k} - \frac{1}{h}\right) V_C$$

when k samples are taken. In the second limiting case where the whole of the variation is due to the variation between sampling units, with no competition, the variance between plots is inversely proportional to the number of units, being V_S/k. In the third limiting case where the whole of the variation is due to variation from plot to plot without any variation between sampling units in the same plot the variance between plots is independent of the number of units taken, being V_P.

The estimates A and B of the analysis of variance can now be interpreted in terms of V_P' and V_S'. We have, except for errors of estimation,

$$B = V_S',$$
$$\frac{1}{k} A = V_P' + \frac{1}{k} V_S',$$

or, in place of the second equation,

$$A - B = k V_P'.$$

In general V_P is likely to be large compared with $(1/h)\ V_c$, and therefore V_P' may be expected to be positive. It will be noticed that the effect of competition cannot be separated from the other sources of variation, but that if $A-B$ is clearly and significantly negative the existence of competition is demonstrated.

The above method of considering the whole of the variation as made up of a number of additive parts enables the more complex cases which occur in practice to be easily comprehended. In the first place in experimental work we are not usually concerned with the total variance from plot to plot of a set of plots, but only the residual variance after treatments, block effects, etc., have been eliminated. This can be represented as the variation due to a set of quantities which are added to quantities representing the effects of treatments, blocks, etc.; otherwise the argument proceeds as before.

Secondly, each plot may be subdivided for sampling purposes, so that, for example, half the samples are taken from each half of the plot, selection being at random within the half-plots. In this case differences which affect the whole of each half-plot are automatically eliminated from sampling error, but appear in experimental error. Here again the extra terms can be easily introduced.

Thirdly, the plots may be subdivided for certain subsidiary treatments. The formal statement in the case of quarter plots is as follows. If V_Q and V_{QC} are the variances of the additional quantities representing the part of the variation which is constant over quarter plots and the part due to competition between quarter plots respectively, and h is the possible and k the actual number of sampling units per whole plot, and we put

$$V_S+V_C=V_S', \; V_Q+V_{QC}-V_C/\tfrac{1}{4}h=V_Q', \; V_P-\tfrac{1}{4}V_{QC}=V_P',$$

the variance between sampling units in the same quarter plot is V_S', that between the means of quarter plots (the part unaffected by whole plot differences) is $V_Q'+V_S'/\frac{1}{4}k$, and that between the means of the whole plots is $V_P'+\frac{1}{4}V_Q'+V_S'/k$.

The interpretation of the estimates of the analysis of variance in terms of these quantities is similar to that already given.

III. THE EFFICIENCY OF SAMPLING.

The amount of information available in an experiment of given design and with a given number of plots is inversely proportional to the error variance per plot. The efficiency of sampling compared with complete harvesting (assumed equivalent to harvesting of all possible sampling units) is therefore given by the ratio of the error variance per plot when all units are harvested to that obtained when the chosen number are harvested. In terms of the notation of the last section this is

$$\frac{V_P' + \frac{1}{h} V_S'}{V_P' + \frac{1}{k} V_S'}.$$

The fractional loss of information L is one minus this fraction.

If when the whole plot is harvested the part of the error variance due to variation between sampling units is a fraction f of the total error variance, *i.e.* if

$$f = \frac{\frac{1}{h} V_S'}{V_P' + \frac{1}{h} V_S'},$$

and if a fraction x of the plot is actually harvested, then the expression for the efficiency may be written

$$1 - L = \frac{1}{1 + f(1-x)/x}.$$

On substituting the estimates A and B from the analysis of variance we obtain

$$L = 1 - \frac{\frac{1}{k}(A-B) + \frac{1}{h} B}{\frac{1}{k}(A-B) + \frac{1}{k} B} = \left(1 - \frac{k}{h}\right) \frac{B}{A}.$$

Clapham(3) and other writers have taken $\frac{1}{k}(A-B)$ instead of

$$\frac{1}{k}(A-B) + \frac{1}{h} B$$

as the estimate of what the experimental error would have been had the whole plot been harvested. This gives $\frac{B}{A}$ for L instead of our expression. They have therefore tended to underestimate the efficiency of the sampling process actually adopted. The difference between the two expressions arises from the fact that they considered it possible to take an infinite number of sampling units, whereas only a finite number are available. The difference is only small if the fraction of the plot sampled is small, but in considering a group of experiments the exact expression should be used, since the difference is always in the same direction.

Since A is usually based on a fairly small number (n_t say) of degrees of freedom the above estimate of L is very variable. The first approximation to its standard error is $\sqrt{2B^2/n_t A^2}$. No great reliance should therefore be placed on the results obtained from any one experiment, but when a whole group of experiments harvested by similar methods is available then the mean loss of efficiency should give a good idea of the general efficiency of the sampling method. Before taking the mean of a set of values of L, however, a correction should be applied. For if we have a group of identical experiments the L's will be biased in a positive direction, differing from the true losses of efficiency by quantities whose mean value is the mean of

$$\frac{2}{n_t}L.$$

The mean of the quantities

$$L' = \frac{n_t - 2}{n_t} L$$

is therefore the appropriate measure of the loss of efficiency from which to judge the adequacy or otherwise of the sampling methods applied to a group of experiments.

IV. THE MATERIAL.

The list of experiments (other than those of the Crop-Weather Scheme) which were harvested by the sampling method and on which it was possible to calculate the sampling errors, is given in Table I. Table II gives the different types of design, and Fig. 1 illustrates the various types of sampling unit employed.

Table I. *List of experiments.*

No.	Year	Place	Crop	Type of sample
1	1929	Rothamsted	Barley	0
2	,,	,,	Oats	0
3	,,	,,	Wheat	0
4	,,	Wellingore	Barley	1*a*
5	1930	,,	,,	1*b*
6	,,	Sparsholt	,,	1*c*
7	,,	Rothamsted	Wheat, barley	1
8	1931	Sparsholt	Barley	1*b*
9	,,	Wye	,,	1*b*
10	,,	Rothamsted	Wheat, barley	1′
11	,,	,,	,,	1′
12	,,	,,	Wheat	1′
13	1932	Wellingore	Barley	2
14	,,	Sparsholt	,,	2
15	,,	Wye	,,	2
16	1933	Wellingore	,,	2
17	,,	Wye	,,	2
18	,,	Rothamsted	Wheat	3

The plots of all the experiments of Table I were approximately square in shape. As far as can be ascertained a single row on each side of a plot or a subplot, and about 1 ft. at each end, was excluded when selecting the sampling units in all experiments except 1, 2 and 3, where 1 yard at each end was rejected. This has been allowed for when calculating the percentage sampled. The processes of selecting the sampling units followed closely those described by Clapham (2). It may be mentioned in passing that this process is not quite correct, since it excludes the possibility of two units being selected from the same set of rows.

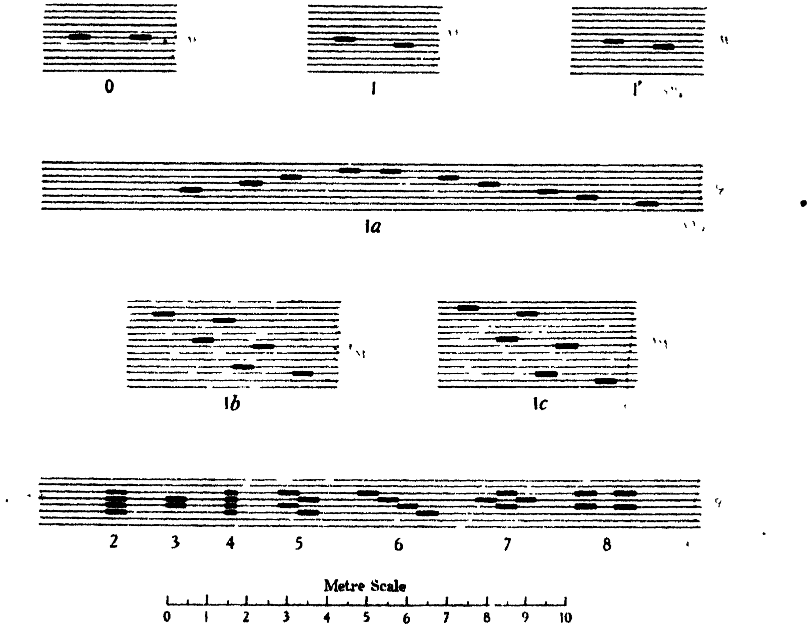

Fig. 1. Types of sampling unit.

In addition to the experiments listed in Table I there are the wheat experiments of the Crop-Weather Scheme. In these experiments the progress of the wheat plant is observed from sowing to harvesting. Eight randomised blocks of two or three varieties are grown, the plots being one drill width wide and 25 metres long. In drilling the centre coulter is blocked, so that each plot is divided into two halves, usually six rows wide. A sampling unit consists of four contiguous quarter-metre lengths from the four central rows of the half-plot (Fig. 1, type 4); the two outside rows are not observed. At each observation date two sampling units from each half-plot are observed, these being a random selection from the 100 possible sampling units. The same method of sampling is used for determining the harvest yields. Thus 2 per cent. of the whole crop is harvested. Sampling errors for yield could be computed in five out of eight of these experiments in 1932–3, and seven out of nine in 1933–4.

Table II. *Designs.*

Exp.	
1	Two 5 × 5 Latin squares, with plots subdivided into four for treatments.
2	Three randomised blocks of sixteen plots each with plots subdivided into two for sampling.
3	Three randomised blocks of eight plots each with plots subdivided into four for varieties and again into two for sampling (variance between sampling subplots not computed). (The varieties were in strips across the three blocks, but the details need not concern us here.)
4	Two randomised blocks of eight plots each.
5, 6, 8, 9, 13, 14, 15	4 × 4 Latin square with plots subdivided into four for treatments.
7, 10	Long-term rotation experiments, experimental errors not immediately available. Plots divided into two for sampling.
11	Long-term rotation experiment. One block of fifteen plots. Plots divided into two for sampling.
12	5 × 5 Latin square, plots subdivided into two for sampling.
16, 17	6 × 6 Latin square.
18	Six randomised blocks of eight plots each, with plots subdivided into two for treatments and again into two for sampling (variance between sampling subplots not computed).

V. BIAS.

Bias cannot arise if proper methods of random selection are adhered to. But it must be remembered that the sampling process determines the yield at the time of sampling of the areas actually sampled. If edge rows are rejected and these yield less than the centre rows the full yield must necessarily tend to be less. Equally if there are losses either on or off the field which affect the full harvesting but not the sampling (such as bird damage, shedding of grain, etc.) these will inevitably introduce discrepancies. For these reasons, and in order to make certain that the sampling processes laid down are actually being carried out, it is of interest to compare the sampling yields with the full harvest yields where these are available.

Table III shows this comparison, which was only available for the first three of the experiments listed in Table I, and for the two experiments at one centre under the Crop-Weather Scheme. The discrepancies in these last two provide a remarkable contrast to the excellent agreement of the grain yields in the first three. The probable explanation of the major part of these discrepancies is failure to carry out the proper random process of selection: the actual sampling units taken at this centre were composed of four single quarter-metres, not necessarily quite contiguous, located anywhere within a random metre length of the four drill rows, this location being entirely at the discretion of the observer.

Table III. *Comparison of mean yields by full harvesting and sampling.*

		Grain cwt. per acre		Straw cwt. per acre	
Exp.	Crop	Full harvesting	Sampling	Full harvesting	Sampling
1	Barley	24·7	24·8	24·5	25·2
2	Oats	13·1	13·8	25·5	22·5
3	Wheat	17·6	17·7	26·7	24·3
1933*	,,	20·5	27·2	40·6	53·6
1934*	,,	16·4	20·4	31·0	36·1

* Crop-Weather Scheme (one centre).

The first three experiments are discussed in detail in Clapham (1, 3) and the last two in Yates (6). Some reference to the discrepancies in straw yields is made in section X of this paper.

VI. VARIATION OF SAMPLING UNITS.

We will now examine the variability from sampling unit to sampling unit within the sampling subplots. Since, however, the size of the sampling unit varies from experiment to experiment the variation will be more comparable if expressed in terms of the standard deviation per metre sampled. Thus if the actual sampling units contain 3 metres the sampling standard deviation will be multiplied by $\sqrt{3}$. The values obtained in the various experiments, expressed as percentages of the mean yields, are shown in Tables IV and V. Table VI gives comparable figures from some preliminary experiments on sampling made by Clapham in 1928 (1). In these experiments a single plot of each variety was sampled; each plot was divided into three equal parts, probably more or less square, and from each part 10 random metre lengths of row were taken.

In Table IV the percentage sampling error per metre for grain (excluding the bracketed figures) ranges from 18·4 to 36·0 for barley, with a mean of 26·6, and from 24·3 to 38·6 for wheat, with a mean of 28·7. The crop-weather values of Table V are somewhat lower, having a mean of 24·5, with a range from 16·6 to 32·2. Clapham's preliminary values, Table VI, are decidedly higher. The figures for straw are very similar.

Differences in the percentage sampling error may arise from various causes, besides inherent differences in the variability of the material.

The larger sampling subplots may be expected to give higher sampling errors. Variation in type of sampling unit will also introduce certain differences: Kalamkar (4) found that in a particular 1/40th acre of wheat examined by him by harvesting every half-metre separately four contiguous half-metre rows (type 2) gave a smaller sampling error than any other type of sampling unit tried. The yields also vary widely and there is no reason to suppose that using percentage sampling errors will completely eliminate the effect of this variation.

Table IV. *Yields and sampling errors—experiments of Table I.*

				Grain		Straw	
Exp.	Place	Type	Sampling subplot area	Yield cwt. per acre	Sampling error % per metre	Yield cwt. per acre	Sampling error % per metre
			Barley.				
1	Rothamsted	1	1/160	24·8	29·8	25·2	30·2
4	Wellingore	1 *a*	1/60	20·2	23·7	18·5	25·8
5	,,	1 *b*	1/200	14·7	33·9	16·8	30·8
6	Sparsholt	1 *c*	1/200	13·9	23·9	13·6	21·1
7	Rothamsted	1	1/80	25·0	30·4	29 1	30·5
8	Sparsholt	1 *b*	1/200	17·2	18·4	28·3	20·3
9	Wye	1 *b*	1/200	22·6	19·4	23·2	20·4
10	Rothamsted	1′	1/80	22·3 [12·0]	32·5 [33·5]	31·0 [19 3]	28·4 [27·8]
11	,,	1′	1/80	20·1	36·0	25·7	33·0
13	Wellingore	2	1/200	30·1	25·7	32·4	29·1
14	Sparsholt	2	1/200	24·9	26·8	26 3	31·4
15	Wye	2	1/200	28·7	24·0	35·6	25·6
16	Wellingore	2	1/120	23·6	24 5	28·4	24·3
17	Wye	2	1/120	26·4	23·3	28·3	21·2
			Mean	22·5	26·6	25·9	26·6
			Wheat.				
3	Rothamsted	1	1/110	17·7	28·6	24·3	32·9
7	,,	1	1/80	19·6 [14·7]	38·6 [44·6]	30·6 [23·3]	37·2 [43·7]
10	,,	1′	1/80	17·4 [11·0]	28·7 [34·9]	34·7 [21·7]	29·3 [31·1]
11	,,	1′	1/80	19·2	25·0	39·3	23·2
12	,,	1′	1/200	18·2	27·0	35·6	24·5
18	,,	3	1/160	23·3	24·3	34·0	23·4
			Mean	19·2	28·7	33·1	28·4
			Oats.				
2	Rothamsted	1	1/80	13·8	32·1	22·5	30·2
*	Newport	4	1/200	—	23·7	—	18·1

* Crop-Weather Scheme.

There are no outstanding differences in the material before us, other than the higher variability in Clapham's preliminary experiments, this difference probably being due partly to the greater area of the sampling subplots and partly to the fact that the sampling unit was a whole metre cut from a single row: a high correlation between parts of the same row would make such a sampling unit relatively inefficient, as Clapham himself concludes.

Table V. *Yields and sampling errors—Crop-Weather Scheme.*

		Grain		Straw	
Year	Place	Yield cwt. per acre	Sampling error % per metre	Yield cwt. per acre	Sampling error % per metre
1933	Boghall	32·8	16·6	64·8	15·8
	Newport	35·3	22·9	53·7	28·1
	Rothamsted	23·6	28·1	36·1	27·7
	Sprowston	25·2	30·0	46·0	27·4
	Seale Hayne	20·3	20·9	37·4	18·7
	Mean	27·4	23·7	47·6	23·5
1934	Boghall	35·7	18·0	59·3	19·8
	Cirencester	32·6	21·8	60·4	21·4
	Newport	43·7	21·0	66·0	20·8
	Plumpton	35·2	29·3	39·6	28·0
	Rothamsted	32·1	28·2	44·2	29·4
	Sprowston	29·2	24·9	36·9	26·2
	Woburn	20·4	32·3	36·1	25·0
	Mean	32·7	25·1	48·9	24·4
	General mean	30·5	24·5	48·4	24·0

Table VI. *Yields and sampling errors—preliminary experiments.*

			Grain		Straw	
Crop	Variety	Sampling subplot area	Yield* cwt. per acre	Sampling error % per metre	Yield* cwt. per acre	Sampling error % per metre
Barley	824	1/50	24·7	32·8	38·1	29·3
,,	Spratt Archer	1/50	26·4	31·3	41·7	33·4
,,	Plumage Archer	3/100	23·7	40·9	34·5	45·5
Wheat	Red Standard	1/15	20·9	44·3	57·4	47·8

* Assuming that rows are spaced 6 in. apart.

Apart from this difference there is some evidence in the barley grain results of high variability at Rothamsted, and this rather than the change in sampling unit may account for the lower values obtained for wheat in the Crop-Weather Scheme than in the Rothamsted experiments.

In the barley results the change of sampling unit from type 1 to type 2 does not give sufficiently large reductions of the sampling errors for these to be evident. The differences obtained by Kalamkar, Table VII, however, are not sufficiently large to be detected in any comparison of the values of Table IV, where in any case different years are involved.

Table VII. *Sampling errors—Kalamkar's experiments.*

Sampling unit Type	Sampling error % per metre Grain
2	30·3
5	35·8
6	33·0
7	34·0
8	36·8

Mean yield grain: 20·2 cwt. per acre. Sampling subplot: 1/240th acre.

That the percentage sampling error is likely to be somewhat larger in low-yielding experiments is shown by the bracketed results of Table IV. In these experiments some of the plots were unmanured: in all cases except one (for barley straw) the unmanured plots had slightly higher percentage sampling errors.

There are only two experiments on oats; these show similar sampling errors to the other crops.

These results show that for wheat or barley the percentage sampling error per metre length sampled has a mean value of about 25 per cent. for small plots (less than 1/100th acre) for both grain and straw. It rarely exceeds 35 per cent. or falls below 18 per cent.

VII. LOSS OF INFORMATION BY SAMPLING.

It has been shown in section III that the loss of information due to sampling is dependent on the percentage sampled, and on the percentage of the total error variance attributable to variation between sampling units when the whole experiment is harvested by sampling methods. Table VIII gives the actual percentages of information obtained for various values of these two quantities, being calculated from the formula of section III.

Table VIII. *Percentage information obtained from various intensities of sampling.*

Percentage sampled (x)	Percentage of total variance due to variation between sampling units when whole plot is sampled (f)									
	0	¼	½	1	2	5	10	20	50	100
¼	100	50	33	20	11	5	2	1	½	¼
½	100	67	50	33	20	9	5	2	1	½
1	100	80	67	50	34	17	9	5	2	1
2	100	89	80	67	51	29	17	9	4	2
5	100	96	91	84	72	51	34	21	10	5
10	100	98	96	92	85	69	53	36	18	10
20	100	99	98	96	93	83	71	56	33	20
50	100	100	100	99	98	95	91	83	67	50
100	100	100	100	100	100	100	100	100	100	100

The efficiency of sampling is clearly largely dependent on the percentage of the total error variance attributable to variation between sampling units. In other words, as common sense would indicate, if most of the experimental variation is due to variation between sampling units then the amount of information obtained is almost proportional to the number of sampling units, whereas if most of the experimental variation is due to other causes a small number of sampling units per plot will suffice to extract the greater part of the information contained in the experiment.

The knowledge of the average percentage sampling error per metre length and the average experimental error per plot, in the experiments under consideration, enables the amount of information lost to be calculated. Taking the percentage sampling error per metre length as 25 per cent. for sampling subplots of 1/200th acre, the percentage standard error of a whole plot of 1/50th acre due to variation of sampling units is $25/\sqrt{530}$ or 1·09 per cent., since there are 530 metre lengths (after deducting 20 per cent. for edges) in 1/50 acre with the coulters set at 6 in. Thus with an experimental standard error of 10 per cent. when the whole plot is harvested the percentage of the total variance due to variation between sampling units is $100\,(1{\cdot}09)^2/10^2$ or 1·2 per cent., and Table VIII indicates that the loss of information with the conventional 5 per cent. sampling is then only 18 per cent. But with an experimental error of 7½ per cent. the percentage of the total variance is 2·1 per cent., giving a loss of information of 29 per cent., and with experimental error of 5 per cent. the percentage of the total variance is 4·8 per cent., giving a loss of information of almost 50 per cent.

Tables IX and X give a list of the sampling and experimental errors of the experiments considered in this paper, together with estimates of what the experimental errors would have been had the whole area been sampled. These last errors are subject to large errors of estimation, but their root mean square (including the negative estimates of variance) will be an unbiased general mean. For the experiments of Table I this has the value of 8·77 per cent. for grain, and 8·98 per cent. for straw. Taking the subplots instead of the whole plots of Exp. 3, of which the plots are exceptionally large, gives 7·75 and 8·64 per cent. respectively. The corresponding figures for the Crop-Weather experiments are 4·75 and 5·14 per cent. Thus 7½ per cent. appears to be a better general estimate for the standard error per plot of cereal experiments than the figure of 10 per cent.

An estimate of the actual percentage of information obtained in each experiment has been worked out by the expression for L' of section III. These estimates are also shown in Tables IX and X. The mean of the values for the grain from the experiments of Table I (using the subplots of Exp. 3) is 68·8 per cent. Thus it appears that in these experiments

Table IX. *Sampling and experimental errors and percentage information—experiments of Table I.*

					Grain				Straw			
					Percentage errors per plot				Percentage errors per plot			
Exp.	Plot area	Percentage sampled (excluding edges)	No. of metres per whole plot	Experimental D.F.	Sampling	Experimental	Non-sampling experimental	Percentage information obtained	Sampling	Experimental	Non-sampling experimental	Percentage information obtained
1	1/40	6·1	32	12	5·53	10·42*	8·94	78·0	5·58	13·56	12·43	86·8
					4·99	6·98	5·03	60·1	5·09	7·58	5·76	64·7
2	1/40	5·7	30	30	5·87	12·36	10·96	80·2	5·51	13·09	11·95	84·4
3	4/55	6·6	96	15	2·92	20·39	20·19	98·3	3·36	16·89	16·57	96·7
	1/55	6·6	24	51	5·84	11·69	10·27	77·2	6·73	14·41	12·90	80·4
4	1/60	5·1	20	7	5·31	6·19	3·40	50·2	5·78	4·51	?	−11·4
5	1/50	6·6	24	6	6·93	7·94	4·26	52·6	6·29	6·98	3·43	49·5
6	1/50	5·6	24	6	4·87	8·76	7·37	80·5	4·31	7·67	6·43	80·1
8	1/50	9·8	36	6	3·07	5·84	5·06	83·4	3·39	7·23	6·47	86·8
9	1/50	9·8	36	6	3·23	4·42	3·18	67·9	3·40	7·53	6·80	87·8
11	1/40	4·0	24	9	5·11	11·76	10·64	86·0	4·73	9·11	7·84	79·9
					7·38	10·79	8·01	65·2	6·75	9·43	6·72	62·0
12	1/100	5·3	12	12	7·79	8·34	3·48	31·2	7·07	8·72	5·36	48·0
13	1/50	7·5	32	6	4·55	6·42	4·70	69·0	5·15	7·41	5·51	70·2
14	1/50	9·3	32	6	4·73	10·73	9·74	88·2	5·54	10·53	9·11	83·3
15	1/50	8·7	32	6	4·24	9·19	8·25	87·0	4·52	11·82	11·00	91·1
16	1/120	5·3	10	20	7·74	7·98	2·64	19·8	7·70	10·69	7·62	55·9
17	1/120	6·3	10	20	7·36	13·50	11·47	75·0	6·70	10·96	8·83	68·4
18	1/40	5·5	32	27	4·44	11·70	10·87	87·4	4·27	15·10	14·52	93·0

* Clapham's value was 5·58.

Table X. *Sampling and experimental errors and percentage information—Crop-Weather Scheme.*

			Grain				Straw			
			Percentage errors per plot				Percentage errors per plot			
Year	Place	Experimental D.F.	Sampling	Experimental	Non-sampling experimental	Percentage information obtained	Sampling	Experimental	Non-sampling experimental	Percentage information obtained
1933	Boghall	7	8·32	8·77	3·04	37·2	7·94	10·09	6·33	56·6
	Newport	4	11·44	21·51	18·29	86·1	14·04	24·02	19·60	83·2
	Rothamsted	12	14·03	16·39	8·70	40·2	13·84	15·42	7·09	34·3
	Sprowston	7	15·01	10·58	?	−40·9	13·69	8·69	?	−73·7
	Seale Hayne	14	10·46	12·35	6·74	39·8	9·33	7·39	?	−33·9
1934	Boghall	7	8·98	10·21	5·03	45·9	9·89	13·07	7·52	59·9
	Cirencester	7	10·91	13·40	7·93	53·6	10·70	12·18	5·53	46·0
	Newport	7	10·52	5·19	?	−187·4	10·41	5·23	?	−176·7
	Plumpton	7	14·63	11·80	?	−7·5	14·01	12·79	?	16·0
	Rothamsted	14	14·08	12·30	?	−9·9	14·71	15·48	5·24	24·1
	Sprowston	14	12·44	12·27	?	13·6	13·11	12·56	?	8·5
	Woburn	14	16·17	16·72	4·82	21·4	12·51	11·75	?	4·8
	Mean		12·25	12·62	—	7·7	12·02	12·39	—	4·1

just about one-third of the information was lost by sampling, assuming full harvesting to be equivalent to sampling the whole area.

In the Crop-Weather wheat experiments only about 10 per cent. of the possible information on varietal differences of grain yield is obtained by the sampling method adopted.

In view of the fact that about one-third of the information available has been lost by harvesting by the sampling method it is of interest to see what would be gained by sampling more extensively. The average percentage sampled (excluding the edges of the plots) was 6·0. We will assume that the mean loss of information, 32·8 per cent.[1] for grain, is representative of this class of experiment. Then, by the formula of section III,

$$1-0{\cdot}328=\frac{1}{1+f\,0{\cdot}94/0{\cdot}06},$$

which gives 0·03116 as the value of f, the fraction of the error variance due to variation between sampling units when the whole plot is harvested.

If double the number of sampling units had been taken, *i.e.* 12 per cent., the fraction of the total information obtained would have been

$$\frac{1}{1+0{\cdot}03116\times 88/12}=81{\cdot}4 \text{ per cent.}$$

Similarly treble the number of sampling units, *i.e.* 18 per cent., would give 87 per cent. of the total information. These values could also be obtained by interpolation in Table VIII.

The additional information that would have been obtained had the amount of sampling been doubled is thus 14·2 per cent. of the total information, or 21·2 per cent. of the information actually obtained. Trebling the sampling, however, only gives a further 6·2 per cent. of the total information, or 9·3 per cent. of the information actually obtained.

VIII. THE WORK INVOLVED IN SAMPLING AND OTHER EXPERIMENTAL OPERATIONS.

The smaller the percentage sampled the larger must be the size of the experiment in order to yield a given total quantity of information. We will now consider the question of the most efficient balance between the percentage sampled and the size of the experiment.

This question is easily answered if the relation between the amount of work required to increase the sampling by a given amount, and that required to increase the size of the experiment by a given amount, is known.

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[1] This value was arrived at before the error in Exp. 1, referred to in the footnote to Table IX, was discovered. The difference from 31·2 is trivial.

In practice this relationship varies considerably from experiment to experiment and is not very easily determinable, but as we shall shortly show considerable changes in the relation do not greatly affect the optimal value of the percentage sampled. Nor is there any great loss of efficiency with even quite considerable deviations from the optimal percentage of sampling.

The following is an estimate of the additional man-hours for each additional plot of about 1/40th acre, over the man-hours that would be needed for commercial farming of the same land:

		Man-hours per plot
Excluding harvesting:	Fertiliser experiments	1·60
	Cultivation experiments	2·02
Full harvesting:	Without stacking	0·73
	With stacking	0·93
Full threshing		0·80

These figures are based on Rothamsted experience and do not include any allowance for machinery used. Nor, of course, do they include any items of work which are constant whatever the size of the experiment.

A high proportion of total time is absorbed by harvesting and threshing. (Clapham's measure of this time, 1·85 man-hours without stacking, is even larger, but probably no deduction was made for the commercial harvesting.) At first sight, therefore, there appears to be a promising field for sampling, but unfortunately the work involved in sampling is even more considerable. Clapham gives 2·19 man-hours per plot as the average time (including the threshing) taken to sample his experiment, in which 24–32 separately located metre lengths were cut. An estimate based on recent experience is as follows:

$$\left.\begin{array}{l}\text{Sampling: } \tfrac{1}{2} \text{ man-hour} \\ \text{Threshing: } 1\tfrac{1}{2} \text{ man-hours}\end{array}\right\} \begin{array}{l}\text{per plot of 16 two-metre} \\ \quad\text{sampling units}\end{array}$$

This agrees closely with Clapham's times.

The threshing is the greatest time consumer. Clearly it should be possible to build a machine which would reduce this time materially. The present machine is only capable of dealing efficiently with small samples of about 2 metres of drill row. There is no need to have more than two independent sampling units per plot (this will give the same number of degrees of freedom for the estimate of sampling error as there are plots), and there does not appear to be any reason why with a suitable machine the man-hours per sampling unit for threshing should be much greater than at present, even with very much larger units.

The experimental threshing of the whole produce of a 1/40th acre plot takes about 1·33 man-hours, and the commercial threshing of the same produce takes about 0·53 man-hour. The difference of 0·80 man-hour represents the limit of what can be expected in the way of machine improvements when dealing with samples. This gives about 0·05 additional man-hour for each 32 metres sampled. Double this, 0·10 man-hour, may be taken as the figure more likely to be attained in practice. To this must be added a constant number of man-hours for each sampling unit, which will include weighing, clearing the machine, etc. This may be taken about 0·1 man-hour per unit.

If sampling is to be increased it may very well be profitable to use larger rectangles of crop than the present four rows by ½ metre. This point will be discussed in a later section. A saving of time will then be effected on location and bagging, and a further saving might be effected• with improved cutting instruments. Thus the rate of increase of work entailed if the percentage sampled is increased might under favourable circumstances and with suitable apparatus be as low as 0·2 man-hour for sampling and 0·1 man-hour for threshing for every additional 32 metres sampled.

We thus have the following numbers of man-hours for sampling the first and subsequent 32 metres (6¾ per cent.) of a 1/40th acre plot:

	First 32 metres	Subsequent 32 metres
Sampling	0·50	0·20
Threshing	0·30	0·10

IX. DETERMINATION OF THE OPTIMAL AMOUNT OF SAMPLING.

We are now in a position to determine the optimal amount of sampling. If Q is a number representing the necessary size of the experiment (equal to unity when the whole experiment is harvested) and S a number representing the relative total number of samples (also equal to unity when the whole of an experiment of unit size is sampled) so that $100S/Q$ is the percentage sampled, the condition that a constant quantity of information is obtained whatever the percentage sampled is equivalent to

$$Q(1-L)=1 \quad \text{or} \quad Q\frac{1}{1+f(Q-S)/S}=1,$$

which reduces to

$$\frac{1-f}{Q}+\frac{f}{S}=1.$$

The graph of this function for $f=0{\cdot}03116$ is exhibited in Fig. 2. The graphs for $f=0{\cdot}2$ and $f=0{\cdot}5$ are also shown for purposes of comparison. The limiting value of S (Q infinite) is in each case equal to f.

As will be seen, for $f=0{\cdot}03116$ there is a rapid reduction in the required size of the experiment with increasing sampling till S equals about 0·15. Q then equals 1·23 and the percentage sampled is therefore $100\times 0{\cdot}15/1{\cdot}22$ or about 12 per cent. Beyond this point the rate of reduction in Q rapidly falls off, so that there is little further gain after S reaches 0·25, at which point Q is 1·11, and the percentage sampled is 22½ per cent.

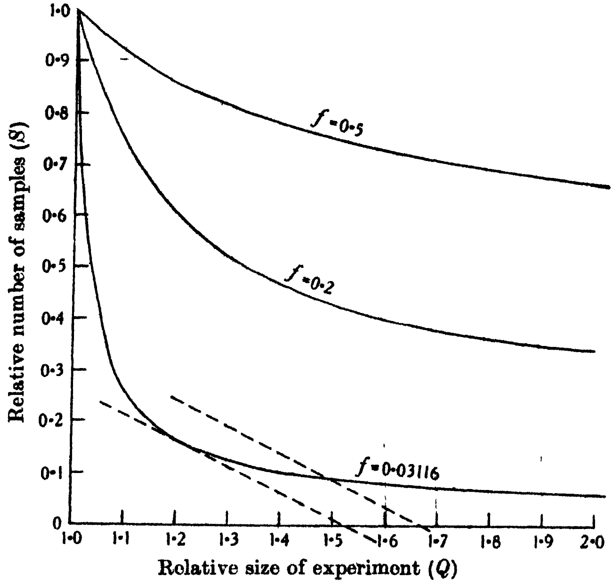

Fig. 2. Relation between number of samples and size of experiment required for a given amount of information.

The amount of sampling is optimal if the total work is a minimum. Only additional work involved in increasing the percentage sampled, or the size of the experiment, need be considered when determining the optimal point. Work which is independent of the size of the experiment, such as moving implements to the field, giving instructions, etc., does not affect the issue.

At the rate of work given above the sampling of the whole plot would take 4·45 man-hours in addition to the constant time involved in any sampling. The additional man-hours for each additional plot excluding harvesting has a mean value of 1·81 man-hours, and to this must be added the constant element of the work involved in sampling the plot, namely 0·50 man-hour, giving in all 2·31 man-hours per additional plot.

The ratio of the additional amounts of work in unit increments of S and Q may therefore under favourable circumstances be as low as 4·45 : 2·31 or approximately 2 : 1. In this case $2S+Q$ should be a minimum. The point on the curve at which this occurs will be given by the point of contact of the tangent parallel to $2S+Q=0$. This tangent is shown dotted in Fig. 2. The point of contact gives almost exactly 12 per cent. sampling.

With the thresher at present available, however, the ratio is probably closer to 4 : 1. The optimal percentage of sampling is then about 9 per cent.

It is important not to exaggerate the loss of efficiency, which is quite small over a considerable range of sampling. The total amount of work involved for the optimal degree of sampling is represented by the point where the tangent cuts the scale of Q, and the amount of work for any other degree of sampling is represented by the point where the same scale is cut by the line parallel to the tangent passing through the corresponding point on the curve. Thus the tangent shown cuts the scale of Q at 1·52, and the parallel line through the point on the curve giving 6 per cent. sampling, namely $Q=1{\cdot}49$, $S=0{\cdot}895$, cuts the scale of Q at 1·67. There is thus 10 per cent. more total work (excluding work independent of the size of the experiment) with 6 per cent. sampling if 12 per cent. is optimum.

It is equally important, however, not to gloss over the very considerable loss of information which occurs when only a small percentage of an experiment is sampled, as compared with the information obtained when the whole experiment is harvested. As we have seen in this series of experiments one-third of the total information is lost, and this implies that as far as the main treatments are concerned the experiments should have been laid down with 50 per cent. more replicates than would be requisite if the whole of the plots were harvested.

One further point should be borne in mind. When several sets of samples are taken from the same experiment, as for example in the study of the growth of a crop, the experiment should be so arranged that the greater part of the variance of the final results is due to variance between sampling units. This requires that a small percentage of a large number of plots is sampled. By this means, although the percentage actually recovered of the total amount of information that the experiment is capable of giving is small, yet the amount of information per sampling unit is a maximum, since plot errors are almost eliminated owing to the large number of replicates.

The experiments of the Crop-Weather scheme are of this type, and the low percentage of the total information recovered is therefore no argument for reducing the size of the experiment. Conversely, if more accurate results were required it might be preferable to keep the same percentage of sampling and increase the number of replicates rather than increase the percentage of sampling on the present number of replicates.

Another type of observation in which the sampling technique is often useful is that in which individual elements within plots are necessarily recorded separately but in which the recording involves comparatively little work, as for example the measurement of the girth of rubber trees. Here, if the constant element of plot variance is at all large (f small), it may still be worth while sampling, even if approximately the whole of the information is required. Referring again to Fig. 2 we see that if f is 0·03116 with 50 per cent. sampling 97 per cent. of the information is obtained; yet half the trees would not require measurement. With f equal to 0·2, on the other hand, all the trees would require measuring if the loss of information were to be negligible.

In sampling for subsidiary quantities the maximum amount of information is frequently not required. Knowledge of the sampling and experimental error variances then immediately enables the required degree of sampling to be determined. In any series of sampling measurements of which the experimenter has no previous experience, it is always well worth while examining critically the first set of measurements to determine the actual accuracy obtained. If the sampling is found to be excessive considerable later work will be saved. Equally if it is inadequate it can be increased, or if this is not practicable the whole programme can be abandoned with little wasted effort.

X. COMPARISON OF SAMPLING AND FULL HARVESTING.

In conclusion we may compare the work involved in optimal sampling with that of full harvesting. With the values most favourable to sampling and 12 per cent. sampling the number of man-hours per plot for sampling and threshing is

$$0{\cdot}50 + 0{\cdot}30 \times 12/6\tfrac{3}{4} = 1{\cdot}03 \text{ man-hours.}$$

This must be multiplied by 1·23 to allow for the additional size of the experiment, and there must be added 0·23 times the amount of work for one additional plot prior to harvest. The total work is therefore

$$1{\cdot}03 \times 1{\cdot}23 + 1{\cdot}81 \times 0{\cdot}23 = 1{\cdot}69 \text{ man-hours.}$$

This is remarkably close to the work of full harvesting per plot, namely 1·73 or 1·53 man-hours according as the produce is stacked or not.

It thus appears that under favourable circumstances sampling for yield involves about the same amount of work as harvesting in full, after allowance is made for the necessary increase in size of the experiment. But with the present methods of sampling the amount of work for sampling is very considerably more, and in experiments where subdivision of the main plots for subsidiary treatments is not required full harvesting appears to be definitely more efficient, judged merely on the basis of labour expended.

There are, however, other advantages of sampling which must be taken into consideration:

(1) In experiments on farms at some distance from the organising station there will probably be no facilities for threshing experimental plots, and sampling then becomes a necessity.

(2) Sampling enables the edges of the plots to be rejected with ease. This is very difficult with full harvesting and is frequently not done, reliance being placed on paths between the plots.

(3) Very small plots are more easily dealt with by the sampling technique. The possible gain in efficiency by any reduction in size of plot made possible by sampling has not been considered here, but may in certain cases be considerable.

(4) Subdivision of plots for subsidiary treatments can be easily effected, since the larger number of replications on these treatments permits of a lower accuracy per plot without reducing the accuracy of the comparisons involved below those of the main treatments.

(5) Losses in the stook and in the stack are avoided. These losses, however, can be kept small if proper precautions are taken.

(6) Weed infestation, plant density, etc., can be estimated with little extra labour.

Against these advantages must be set the following disadvantages:

(1) Harvesting of a lodged crop by sampling is almost impossible.

(2) The ritual of "random selection" must be carefully observed if biased results are to be avoided (6), and workers capable of carrying out the procedure satisfactorily are not always available.

(3) Sampling is generally admitted to be a tedious operation.

(4) Sampling yields do not carry the same conviction in certain

quarters as the yields from full harvesting. The same applies to the yields of the very small plots which sampling makes possible.

Finally it should be remembered that our assessment of the relative advantages of sampling and full harvesting has been based on labour requirements only. In practice many other factors, such as machinery, availability of land, distribution of work, etc., must be taken into consideration, but the method of assessment is the same; and though the exact calculation in any particular case might be very laborious, so that the final decision is necessarily based on empirical judgment, yet an exact knowledge of the relative efficiency in the simpler cases is likely materially to improve such judgment.

The above comparison of sampling and full harvesting is based on the assumption that there are no extraneous errors which affect full harvesting but do not occur in sampling, so that sampling of the whole plot may be considered as equivalent to full harvesting. There is little direct evidence on this point, since only three of the eighteen experiments were harvested completely. The excellent agreement of the yields of grain in these experiments (Table III) indicates that there was in fact little loss from the stooks, so that there could scarcely be any increase of error from this cause.

Table XI. *Comparison of error variances of full harvesting and sampling (when the whole plot is sampled).*

		Grain				Straw			
Exp.	Correlation of residuals (grain)	Sampling	Full harvesting	Difference		Sampling	Full harvesting	Difference	
1. Barley	0·744	79·7	71·6	− 8·1	±37·2	154·5	100·8	−53·7	±62·5
	0·654	25·3	14·2	−11·1	±18·2	33·2	39·2	+ 6·2	±22·1
2. Oats	0·631	120·1	122·5	+ 2·4	±39·0	142·7	62·4	−80·3	±28·7
3. Wheat*	0·371	104·3	93·7	−10·6	±30·7	165·3	99·8	−65·5	±30·4

* Subplots (1/55th acre).

The only other possible source of error in the grain appears to be that due to edge effects. Table XI shows the comparison of the experimental errors obtained from the full harvesting and the errors that would have been obtained had the whole of each plot been sampled. The agreement in the case of grain is excellent, the differences being in every case less than their standard errors (calculated by the method given by Wishart and Clapham (5)).

In the case of straw the full harvesting shows decidedly lower errors in three out of the four sets of results, the last two differences being significant. (The correlation between the residuals has only been calculated for the grain, and the standard errors for straw are based on the correlations of the grain.) Clapham himself noted the differences in the case of the oats experiment, and attributes it to weeds, which were included in the full harvesting but partially discarded in the sampling, and to variation in length of straw cut in the sampling. The possibility of eliminating weeds or clover from the straw yields is an advantage of sampling which is sometimes important in that weeds are not always thoroughly dried out when the straw is weighed.

This is vividly illustrated by the yield for the four-course rotation at Rothamsted in 1930. According to the published results the mean yields (in cwt. per acre) were as follows:

	Grain	Straw	Grain/Straw
Treated plots	20·1	32·5	0·618
Untreated plots	11·8	32·3	0·365

This would be a very remarkable result, if true. The experiment was partially sampled (the sampling was abandoned because the crop was badly lodged). The grain yields agreed reasonably well, but the straw yields, while agreeing well in all but one of the treated plots, showed a mean sampling yield on the five untreated plots which were sampled of only 17·6 cwt., as compared with a mean yield of 29·7 cwt. for the same plots by full harvesting. The former figure gives a sampling grain/straw ratio of 0·602.

Enquiry showed that the explanation lay in the very heavy clover crop in the undersown ley, which was considerably more dense on the untreated plots and was still green when the straw was weighed. It would appear that in experiments of this nature, at least, and in other experiments which are very weedy, definite estimation of the amount of clover, grass and weeds must be made if the yields of straw from full harvesting are to have any meaning.

XI. GAIN BY SUBDIVIDING PLOTS FOR SAMPLING.

In most of the experiments under consideration the main plots were subdivided either for subsidiary treatments or in order to increase the precision of the sampling. Both types of experiment can be used to determine how far the precision of the sampling is in fact really increased by this procedure.

We will consider the case of subdivision into quarters. Using the notation of section II we have the following analysis of variance form for all the sampling units of a single plot:

	Degrees of freedom	Sum of squares	Mean square
Between sampling units within quarter plots	$h-4$	$(h-4)\,V_S'$	V_S'
Between quarter plots within plots	3	$\frac{3}{4}hV_Q'+3V_S'$	$\frac{1}{4}hV_Q'+V_S'$
Between sampling units within plots	$h-1$	$\frac{3}{4}hV_Q'+(h-1)\,V_S'$	$\frac{\frac{3}{4}h}{h-1}\,V_Q'+V_S'$

The mean square of the last line represents the variance between sampling units when there is no subdivision. Lack of subdivision has therefore increased the effective sampling variance by the fractional amount

$$\frac{3h}{4(h-1)} \cdot \frac{V_Q'}{V_S'}.$$

Replacing V_Q' and V_S' by their estimates in terms of the mean squares, C and B say, with k sampling units per whole plot this fraction becomes

$$\frac{3h}{k(h-1)} \cdot \frac{C-B}{B}.$$

In general with subdivision of each plot into q parts the fraction is

$$\frac{(q-1)h}{k(h-1)} \cdot \frac{C-B}{B}.$$

If the possible number of sampling units h is large this is approximately equal to

$$\frac{q-1}{k} \cdot \frac{C-B}{B}.$$

Tables XII and XIII set out the values of this fraction for those experiments in which there was subdivision of the plots. As will be seen in most of the experiments the increase in sampling variance (which of course is greater, often considerably greater, than the resultant loss of information on the experimental results) would be quite small, of the order of 5 per cent., but a few of the values are considerably higher, the greatest being over 50 per cent. These, however, are associated with experiments in which the ratio of the sampling to the experimental error was small, so that the extra gain in sampling precision by subdivision produces less gain in total information than might appear at first sight.

The reason these gains are so small is that in most of the experiments in question the number of sampling units per whole plot was quite large, so even if there had been no restriction that equal numbers of units should be taken from each subplot random selection would have given substantially equal distribution. If sets of l of the original sampling units are taken to form new sampling units, then provided there is no correlation within these sets the percentages of Tables XII and XIII must be multiplied by the fraction $l(h-1)/(h-l)$, or roughly, when l is small compared with h, by l. Thus, in Exp. 1*a*, 4-metre units might have been used (2 units to each subplot), instead of the actual

1-metre units; in this case about 20 per cent. more sampling units would have been required if the units had been selected at random from the whole plot instead of equally from the four quarters.

Table XII. *Percentage increase in sampling variance when there is no subdivision of plots for sampling. Experiments of Table I.*

Exp.	Sub-division of plots into	Area of subplots (acre)	No. of sampling units per whole plot	Percentage increase Grain	Percentage increase Straw	Notes
1*a*	¼	1/160	32	5·1	7·6	
1*b*	¼	1/160	32	1·3	3·1	
2	½	1/80	30	10·5	6·8	
5	¼	1/200	8	9·7	15·0	
6	¼	1/200	8	32·4	14·3	
7	½	1/80	30	3·4	− 0·8	Wheat, treated plots
	½	1/80	20	− 1·1	− 1·7	Wheat, untreated plots
	½	1/80	30	8·0	8·8	Barley, treated plots
8	¼	1/200	12	19·7	88·3	
9	¼	1/200	12	1·8	26·9	
10	½	1/80	30	− 1·5	0·4	Wheat, treated plots
	½	1/80	20	4·8	4·4	Wheat, untreated plots
	½	1/80	30	1·2	0·5	Barley, treated plots
	½	1/80	20	4·0	3·0	Barley, untreated plots
11	½	1/80	24	3·1	4·1	Wheat
	½	1/80	24	3·0	0·0	Barley
12	½	1/200	12	5·6	9·2	
13	¼	1/200	16	7·1	23·7	
14	¼	1/200	16	50·1	26·7	
15	¼	1/200	16	42·8	57·1	

Table XIII. *Percentage increase in sampling variance when there is no subdivision of plots for sampling. Experiments of Crop-Weather scheme.*

Year	Place	Percentage increase Grain	Percentage increase Straw
1932–3	Boghall	9·2	15·8
	Seale Hayne	12·4	21·5
	Newport	22·7	9·4
	”	4·5	5·6
	Rothamsted	17·8	16·7
	Sprowston	− 17·3	− 14·9
	Woburn	− 6·9	8·4
1933–4	Boghall	− 13·1	− 8·0
	Cirencester	32·2	17·8
	Newport	− 5·5	− 0·1
	Rothamsted	13·5	35·8
	Sprowston	− 12·5	− 15·0
	Woburn	− 5·0	− 3·6

The arrangement of the sampling procedure so that equal amounts are sampled from the four quarters of the plot is therefore likely to be of considerable value if a few large sampling units are taken of the type suggested in the next section.

XII. SIZE OF SAMPLING UNIT.

Only two independent sampling units from each plot are required, and therefore the greater the percentage sampled the larger can be the individual sampling units. Little will be gained, however, if the sampling units are of a complex type, since the labour of location and cutting will be much the same as with smaller independently located units, and the task of threshing separately a number of small units can always be avoided by bulking into two composite units by random selection (with due regard to imposed restrictions). The chief interest is therefore in units of a simple pattern, which can be easily located, and, given suitable implements, easily harvested.

Using Kalamkar's data, we have tested the efficiency of various sampling units of rectangular shape. The results are set out in Table XIV, where the percentage sampling error per metre and the efficiency relative to Kalamkar's sampling unit (½ metre × 4 rows) are given. As will be seen the units which involve any great length of the same row are very inefficient compared with units of the same size cutting across many rows. The most interesting feature of the results is that strips cutting right across the plot have a relative efficiency of no less than 72 per cent. This suggests that the real solution of harvesting cereals by the sampling method may lie in the evolution of some portable implement which will expeditiously cut lanes about a half-metre wide across the plots. Four such samples from a square 1/40th acre plot (two from each half-plot) would include about 22 per cent. of the non-rejected area and would thus be equivalent to a sampling of 16 per cent. with units of ½ metre × 4 rows. In an experiment of about twenty-five plots the total weight of the samples would be of the order of 7 cwt., which is transportable in a small van or trailer.

Table XIV. *Sampling errors and relative efficiency of large sampling units.*

Sampling unit	Metre length of row	Sampling error % per metre	Relative efficiency
6 metres × 4 rows	24	65·1	22
3 metres × 4 rows	12	53·1	32
1 metre × 4 rows	4	37·2	66
½ metre × 72 rows	36	35·8	72
½ metre × 24 rows	12	31·9	90
½ metre × 12 rows	6	40·2	57
½ metre × 8 rows	4	35·7	72
½ metre × 4 rows	2	30·3	100
1 metre × 8 rows	8	44·9	45
6 metres × 1 row	6	48·1	40
Single ½ metre	½	31·3	94

Before definitely recommending such a procedure, however, this type of sampling unit should be tried out on further data similar to Kalamkar's. Clearly many types of variation may be met with, and it would be rash to base any final conclusions on the results of a single plot; in this connection it may be noted that Kalamkar's plot showed no evidence of any fertility gradient. The shape of the plot is also of material importance, and with long narrow plots the number of sampling units would clearly require to be increased.

XIII. SAMPLING FOR THE RATIO OF GRAIN TO TOTAL PRODUCE.

Many observers have commented on the close correlation which exists between the yields of grain and straw from different parts of a field. If such a correlation exists over different parts of the same plot quite a small sample will be required to determine the proportions of grain and straw.

We now propose to consider the efficiency of the process of weighing the whole of the total produce, and of sampling for the proportions of grain and straw. The sampling results of some of the experiments of Table I will be used.

To make an estimate of the efficiency the variance of the ratio of grain to total produce within sampling subplots will be required. This ratio might be worked out for each sampling unit and an ordinary analysis of variance performed. This, however, would be laborious, and the variance was therefore computed from the variances and covariance (within sampling subplots) of the grain and the total produce. If the grain yield of a sampling unit is denoted by g, the total produce by t, and their ratio by r, with corresponding means $\bar{g}$, t, and $\bar{r}$, then as a first approximation

$$\frac{1}{\bar{r}^2} V(r) = \frac{1}{\bar{g}^2} V(g) + \frac{1}{\bar{t}^2} V(t) - \frac{2}{\bar{g}\bar{t}} \operatorname{cov}(tg).$$

The covariance of the grain and total produce was found for nine of the experiments of Table I. Its values and the values of $V(g)$ and $V(t)$, expressed on a percentage basis, are shown in Table XV, together with the values of $V(r)$, similarly expressed.

The table also shows the sampling standard errors of the ratio, expressed in terms of a single metre of drill row, on the assumption that the metres within each sampling unit vary independently. These errors are in fact the square roots of the product of $100^2\, V(r)/r^2$ and the number of metres per sampling unit.

Table XV. *Sampling for ratio of grain to total produce.*

Exp.	Sampling unit metres	Percentage variances and covariance				Sampling error % per metre	Relative efficiency
		$\frac{100^2\,V(g)}{g^2}$	$\frac{100^2\,\mathrm{cov}(gt)}{\bar{g}t}$	$\frac{100^2\,V(t)}{t^2}$	$\frac{100^2\,V(r)}{r^2}$		
8	3	113·1	98·3	111·5	28·0	9·16	4·0
9	3	124·9	117·6	121·5	11·2	5·80	11·2
12	1	773·5	713·0	683·2	30·7	5·54	25·2
13	2	331·3	334·1	359·0	22·1	6·65	15·0
14	2	358·7	352·1	389·0	43·5	9·33	8·2
15	2	287·7	280·8	293·6	19·7	6·27	14·6
16	2	299·5	264·2	266·7	37·8	8·69	7·9
17	2	270·6	242·0	232·0	18·6	6·10	14·5
18	1	591·2	539·8	534·5	46·1	6·79	12·8

If the weight of the total produce of each plot were accurately known the additional percentage variance of the grain due to sampling for the proportion of grain would be derivable from the percentage variance of r, being merely this variance divided by the number of sampling units per plot.

It is immediately apparent that the sampling is much more effective if used merely to determine the ratio of grain to total produce.

The ratio of the percentage variance of g to that of r, also given in Table XV, is a measure of the relative efficiency per sampling unit of the two methods. The ratios range from 4·0 to 25·2.

The mean percentage standard error per metre length of sample is 7·14 for the ratio of grain to total produce, as compared with the corresponding mean of 23·9 for the grain yields of the same experiments. It thus appears that on the average about ten times as many sampling units are required to attain the same precision when sampling for yield as are required when sampling for the ratio of grain to total produce only. In a 1/40th acre plot there are about 500 metres of drill row. A sample of 2 per cent. of this will give 10 metres of drill row, which on the basis of the above results will have a percentage sampling variance of $7{\cdot}14^2/10$ or 5·1. With a percentage experimental error variance of $7{\cdot}5^2$ or 56·2 the fraction of information obtained is $56{\cdot}2/(56{\cdot}2+5{\cdot}1)$ or 92 per cent.

Since the produce of each plot has to be kept separate for weighing there is no advantage in sampling the standing crop other than possible convenience in the selection of the samples. Sampling from bulk is notoriously difficult, but with proper precautions an unbiased sample can be obtained.

A general method of sampling any material in bulk is to divide the whole of the batch of material into a number of parts, and select one or more of the parts at random, if necessary repeating the process of subdivision. This process effectively prevents bias, indeed it appears to be the only process which will certainly do so, and at the same time it allows the experimenter full scope to equalise the different parts as much as possible. Grab sampling, whatever its nature, may introduce considerable biases. On the other hand, grab sampling is easy to carry out, and may be the only practical method of dealing with large amounts of material. It should be more widely realised that such sampling is likely to be biased, but that its claim to objective reality can be substantiated (or disposed of) by regular supplementary sampling of the above type performed on a small fraction of the batches of material. Much of the routine sampling ordinarily performed for chemical quality determinations is open to criticism on this ground. Great care is taken to ensure accuracy and freedom from bias in the laboratory subsampling, and sometimes to determine the variation between sampling units, quite regardless of the fact that the original samples may be considerably biased.

To sample 2 per cent. of the produce for the ratio of grain to total produce a division into ten parts might be made, and two of these selected at random. Each of the selected tenths might then be divided into ten further parts, and one from each ten selected at random. The difference between the two selected hundredths would provide an estimate of sampling error. There would, however, be no separate measure of the sampling errors introduced by the second division. To obtain this two two-hundredths would have to be selected from each tenth.

When sampling for the ratio of grain to total produce there is one further point which should be borne in mind, namely the loss of moisture in the sample between harvesting and threshing. If the grain and the straw lose moisture in different proportions then the value of the ratio will clearly be affected. In such cases it is impossible to determine the proportions by weight of grain and straw in the total produce when harvested, but if the sample units are weighed at harvest and again at threshing the yields of dry grain and dry straw can be accurately determined by making an allowance for loss of moisture from the total produce. This adjustment is likely to be of importance if there is a considerable volume of green weeds or clover in the harvested crop. Clearly weeds and clover must not be eliminated from the samples unless they are also eliminated from the total produce.

In view of the fact that the efficient sampling procedure when sampling for the ratio of grain to total produce is likely to be widely different from that adopted when sampling for yield there is no point in discussing the present results in more detail here. The figures given above are sufficient to show that this type of sampling is capable of giving very precise results with very small samples. It remains to develop an efficient field technique.

XIV. SUMMARY.

The estimation of the experimental yields of cereal crops by sampling methods is considered in the light of results of eighteen experiments which were harvested by these methods at Rothamsted and its associated centres. The harvesting results of the Crop-Weather series of experiments are also included.

A preliminary discussion of the interpretation of the analysis of variance as applied to sampling results is given, and an expression is found for the loss of information arising out of sampling. The results of the discussion are applicable to all types of sampling carried out on replicated experiments.

The sampling and experimental errors of all the experiments have been calculated. The variation between sampling units in the same subplot (usually between 1/100th and 1/200th acre) was found to be such as would arise if each metre length of row had a standard deviation (about the plot mean) of the order of 25–30 per cent. of the mean yield.

In the main series of experiments the mean loss of information on the main treatment comparisons due to sampling was found to be 31 per cent.

The method of determining the optimal percentage of sampling is described. It is shown that with the present apparatus about 9 per cent. may be considered optimal, but that little loss of efficiency (in terms of total work) arises from the current practice of 6 per cent. sampling. A comparison between sampling and full harvesting is also made.

The gain due to subdivision of the plots for sampling is considered, and it is shown that this is advantageous.

The possibility of simplifying the sampling procedure by using larger rectangular samples is investigated, using Kalamkar's data, and it is shown that so far as his particular plot is concerned strips a half-metre wide cut right across the plot form quite efficient sampling units.

The alternative of sampling for the ratio of grain to total produce only, and weighing the total produce of the plot, is discussed in the light of the results of these experiments. The method is found to be capable of giving very much more precise results than the ordinary procedure;

even with as little as 2 per cent. sampling the loss of information is of the order of 10 per cent. only. It is concluded that this method is likely to be of considerable value.

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(*Received June 20th*, 1935.)

NUTRITIVE VALUE OF LUCERNE.

IV. THE LEAF-STEM RATIO.

By H. E. WOODMAN, M.A., Ph.D., D.Sc.
and R. E. EVANS, M.Sc., Ph.D.
(*The Animal Nutrition Research Institute, School of Agriculture, Cambridge University.*)

INTRODUCTION.

In this publication, which brings to a close the account of the Cambridge investigations into the nutritive properties of the lucerne crop as grown under English conditions, are brought together the numerous data that have been accumulated during the course of the work concerning the ratio of the amount of leaf to stem in the lucerne crop at different stages of growth, and the separate composition of the leafy and stemmy fractions. The results brought forward in previous publications (1, 2) have led to the belief that certain peculiar features of the chemical composition of lucerne, such as the increased protein content that characterises the later seasonal cuts of the crop, are to be attributed to variations in the value of the leaf-stem ratio. On account of the early development of fibrous character in the stems of the plants, it is probable that, in a much higher degree than is the case with grass, the leaf-stem ratio is the factor which determines the nutritive value of lucerne. With this in mind, attention has been devoted to studying the problem of the leaf-stem ratio in relation to variety, since manifestly, other factors being equal, the variety of lucerne to be preferred is that which yields the highest weight of leaf per unit weight of crop.

Apart from its interest to the plant biochemist and the farmer, however, the work has an important bearing on the manufacture of lucerne-leaf meal, a more valuable feeding product than lucerne meal, since in its production a large proportion of the coarse, fibrous stems is excluded (3). Although lucerne-leaf meal is made in large quantities in the United States, its manufacture has not as yet been introduced into this country, and before this can be done with reasonable safety, it is desirable to know whether the reduction in yield of meal per acre of lucerne, consequent on the rejection of the stems, is compensated by the enhancement of the feeding value of the product. The information given in this publication, therefore, should be useful in forecasting the economic possibilities of utilising the lucerne crop for the manufacture of the leaf meal instead of the whole meal.

THE PRESENT INVESTIGATION.

The data recorded in this paper were obtained from investigations of two separate crops of lucerne: (1) a one-year-old stand (variety Provence, sown in 1932) growing on a light gravelly soil on the Howe Hill University Farm at Cambridge; (2) a two-year-old stand (variety Provence, sown in 1931) situated on heavy "skirt" land at Willingham, Cambridgeshire. In the case of this second crop, the studies of the leaf-stem ratio were continued over two seasons (1933 and 1934).

For a detailed description of both crops the reader is referred to an earlier publication (2), in which is also described how the Howe Hill crop suffered, during the second half of the season, under the influence of the summer drought of 1933, whereas the Willingham crop, on the heavy, water-retentive soil, displayed a remarkable capacity for sustaining a fresh, green growth despite the adverse conditions.

The winter of 1933 was unusually dry, and although the rains of March and April were heavy enough to give an abundant, healthy growth of lucerne during the early part of the 1934 season, drought and heat were again prevalent throughout the summer and autumn. Work was discontinued on the Howe Hill area during this second season, and measurements were confined to the more drought-resistant Willingham crop. It should be emphasised that all the records to be discussed in this communication were obtained from plots on which the lucerne plants were showing fresh, verdant growth. Plots which were situated on drought-sensitive areas, as revealed by the manner in which the plants developed a wilted or stunted character during the droughty spells, were excluded from the trials.

THE LEAF-STEM RATIOS.

In Table I are recorded the results of the determination of the leaf-stem ratios on the samples of lucerne from the plots submitted to systematic cutting at the three different stages of growth. The determinations were carried out on large representative samples by stripping the leaves, together with the tender leaf stalks, stipules and growing heads, from the main plant stems. In the case of the flowering crop, the actual flowers were included in the leaf fraction (see Table VI for the analysis of a sample of lucerne flower). The separation in every case was carried out in such a manner as to include in the leaf sample every part of the plant that would be likely to find its way into the lucerne-leaf meal when the crop was being utilised for the manufacture of this product. The amount of crop dealt with was such as to give 600 gm. or more of leaf, the whole of this fraction and the accompanying stem being used in the dry-matter determinations, which in both cases were carried out in duplicate.

Table I. *Summary of leaf-stem ratios.*

System of cuts	No. of cut	Date of cut	Height of crop in.	% dry matter in		Leaf dry matter as % of total dry matter	Stem dry matter as % of total dry matter	Leaf-stem ratio
				Leaf	Stem			
(1) Howe Hill, Season 1933 (variety Provence).								
Pre-bud	1	Apr. 28	16	17·20	19·31	58·26	41·74	1·40
,,	2	May 25	16	16·80	10·64	60·18	39·82	1·51
,,	3	June 23	15	19·29	15·19	57·20	42·80	1·34
,,	4	July 17	15	20·33	16·41	62·31	37·69	1·65
Bud	1	June 1	34	24·50	22·62	44·54	55·46	0·80
,,	2	,, 26	22	17·10	13·08	46·06	53·94	0·85
,,	3	July 25	21	20·50	19·93	49·80	50·20	0·99
Flower	1	June 9	42	24·50	24·48	42·40	57·60	0·74
,,	2	July 20	29	23·50	25·43	46·54	53·46	0·87
(2) Willingham, Season 1933 (variety Provence).								
Pre-bud	1	May 4	13	20·00	16·19	58·50	41·50	1·41
,,	2	,, 29	14	17·50	11·62	61·40	38·60	1·59
,,	3	June 28	12	20·29	18·70	59·05	40·95	1·44
,,	4	July 19	12	21·10	17·60	68·23	31·77	2·15
,,	5	Aug. 16	8	26·40	27·73	65·02	34·98	1·86
Bud	1	June 2	24	25·33	23·75	48·41	51·59	0·94
,,	2	July 7	21	26·91	24·32	51·43	48·57	1·06
,,	3	Aug. 12	26	24·33	26·39	56·15	43·85	1·28
Flower	1	June 19	40	24·00	26·65	42·10	57·90	0·73
,,	2	July 31	30	25·33	30·21	51·70	48·30	1·07
,,	3	Sept. 1	18	24·50	26·50	56·14	43·86	1·28
(3) Willingham, Season 1934 (variety Provence).								
Pre-bud	1	May 4	17	19·44	12·60	57·27	42·73	1·34
,,	2	,, 31	13	20·11	13·21	58·12	41·88	1·39
,,	3	June 22	14	20·88	14·92	57·14	42·86	1·33
,,	4	July 16	13	23·10	18·47	60·00	40·00	1·50
,,	5	Aug. 9	10	21·11	18·33	62·07	37·93	1·64
,,	6	Sept. 4	8	21·80	17·51	70·75	29·25	2·42
Bud	1	May 31	27	24·80	22·18	43·24	56·76	0·76
,,	2	July 5	25	25·93	26·37	45·51	54·49	0·84
,,	3	,, 25	15	23·93	19·80	55·30	44·70	1·24
,,	4	Aug. 22	18	24·76	22·60	59·40	40·60	1·46
Flower	1	June 22	42	26·00	27·22	37·00	63·00	0·59
,,	2	July 25	30	25·86	27·02	48·82	51·18	0·95
,,	3	Aug. 29	24	22·69	24·31	52·67	47·33	1·11

Comments on Table I.

It will be noted that the stems of lucerne frequently contain a higher percentage of moisture than the leaves. This is almost invariably the case with the crop in the pre-budding stage, the difference becoming very marked in the second cut. With the advent of the bud stage of growth there is still, in the case of eight of the ten cuts, this distinction in moisture content, although it is not so pronounced as at the earlier stage of growth. The position is reversed by the time the crop has come into flower, the moisture content of the stems now being lower than that of the leaf. It should be pointed out that these distinctions were observed when the crop was entirely free from superficial moisture.

The data show very clearly how the proportion of leaf in lucerne falls off as the crop advances in maturity. This is best seen by comparing the results in Table II for the first cuts of the different lucerne crops at the pre-budding, budding and flowering stages of growth. It may be observed, however, that the difference between the budding and flowering crop is not so great as between the budding and pre-budding; that is to say, the rate of decrease of leafiness is greater before budding than after.

Table II. *Showing falling-off of leafiness in lucerne crop with increasing maturity.*

	Leaf dry matter as % of total dry matter		
	Howe Hill (1933)	Willingham (1933)	Willingham (1934)
First cut from crop at pre-budding stage	58·3	58·5	57·3
,, ,, budding stage	44·5	48·4	43·2
,, ,, early-flowering stage	42·4	42·1	37·0

If the results in Table II are considered in conjunction with the data for the separate composition of the leaf and stem fractions (see Table VI), no difficulty is experienced in explaining the fall in protein content, and corresponding rise in fibre content, which take place as the crop passes through the successive phases of growth up to flowering (1). Further, when the lignified character of the fibre in the lucerne stems at the stages of both bud and flower (2) is also taken into account, it would be anticipated that these changes must be accompanied by a pronounced running-off in the feeding value of the crop. This has already been shown to be the case, the starch equivalent, on the basis of 100 lb. dry matter, having been found to be 59·8, 51·5 and 42·5 lb. respectively for the first cuts of lucerne at the pre-budding, budding and flowering stages (2).

The leaf-stem ratios recorded in Table I leave no doubt as to the correctness of the conclusion that the later cuts in the season display a distinctly leafier character than the early cuts. This is the case whether the cuts are made systematically at the pre-budding, budding or flowering stage of growth and accounts mainly for the observed higher protein content of the late-seasonal cuts. The effect is such as to give rise to a number of peculiar findings, which may best be illustrated by reference to the leaf-stem ratios for the cuts from the Willingham crop in the 1934 season. Thus, the fourth and final cut from the plot scythed systematically at the bud stage (leaf-stem ratio = 1·46) contained a higher proportion of leaf than any of the first three cuts from the plot cut systematically at a stage prior to bud formation (leaf-stem ratios varying from 1·33 to 1·39). Again, the final cut from the plot submitted to systematic cutting at the early-flowering stage (leaf-stem ratio = 1·11) was leafier than either of the first two cuts from the plot cut at the stage of bud (leaf-stem ratios = 0·76 and 0·84).

It has been pointed out in a previous communication (2) that the early-seasonal growth of lucerne progresses more slowly, from the time standpoint, through the successive stages of growth, and therefore comes up to flower more tardily, than the later growths. This behaviour is obviously responsible for the greater heights and yields that characterise the first cut of the season (see Tables I and III) and may also explain why this cut displays a greater development of stem, in relation to leaf, than is shown by the later cuts. The farmer in this country frequently takes the first cut for hay before the crop has actually come into bloom. By this means he is more assured of securing the maximum possible number of later cuts and also obviates the possibility of the first heavy growth becoming "laid" and difficult to cut, a contingency that might sometimes arise if he waited until the first growth had come into flower. From the standpoint of quality of the hay, the cutting of the first growth in fairly advanced bud appears to be sound, since the results of the present studies of leaf-stem ratios show that to delay too long before taking the first cut involves the danger of a greater reduction in the percentage of leaf in the crop, and consequently in the feeding value of the hay, than would be caused by a similar delay in the later part of the season.

Since the yields of weed-free lucerne were determined at the times when the various cuts were made, it is possible, with the help of the data in Table I, to calculate in every case the separate yields of leaf and stem in terms of lb. dry matter per acre of crop. The results are recorded in Table III.

Table III. *Separate yields of leaf and stem, in terms of lb. dry matter per acre, from lucerne crop (variety Provence) submitted to systematic cutting at three different stages of growth.*

			Yield of			
System of cuts	No. of cut	Date of cut	Leaf lb. dry matter per acre	Stem lb. dry matter per acre	Total lucerne lb. dry matter per acre	Mean leaf-stem ratio over whole season
		(1) Howe Hill, Season 1933.				
Pre-bud	1	Apr. 28	1061	760	1821	
„	2	May 25	605	400	1005	
„	3	June 23	543	407	950	
„	4	July 17	378	229	607	
Totals for four cuts			2587	1796	4383	
Totals expressed as percentages			59·02	40·98	100	1·44
Bud	1	June 1	1788	2226	4014	
„	2	„ 26	1017	1192	2209	
„	3	July 25	824	830	1654	
Totals for three cuts			3629	4248	7877	
Totals expressed as percentages			46·07	53·93	100	0·85
Early flower	1	June 9	2189	2974	5163	
„	2	July 20	1244	1428	2672	
Totals for two cuts			3433	4402	7835	
Totals expressed as percentages			43·82	56·18	100	0·78
		(2) Willingham, Season 1933.				
Pre-bud	1	May 4	1046	742	1788	
„	2	„ 29	418	262	680	
„	3	June 28	576	400	976	
„	4	July 19	263	122	385	
„	5	Aug. 16	90	48	138	
Totals for five cuts			2393	1574	3967	
Totals expressed as percentages			60·32	39·68	100	1·52
Bud	1	June 2	1562	1665	3227	
„	2	July 7	1012	956	1968	
„	3	Aug. 12	661	517	1178	
Totals for three cuts			3235	3138	6373	
Totals expressed as percentages			50·76	49·24	100	1·03
Early flower	1	June 19	1737	2388	4125	
„	2	July 31	1315	1229	2544	
„	3	Sept. 1	495	387	882	
Totals for three cuts			3547	4004	7551	
Totals expressed as percentages			46·97	53·03	100	0·89
		(3) Willingham, Season 1934.				
Pre-bud	1	May 4	1044	778	1822	
„	2	„ 31	659	475	1134	
„	3	June 22	376	282	658	
„	4	July 16	347	232	579	
„	5	Aug. 9	214	131	345	
„	6	Sept. 4	125	52	177	
Totals for six cuts			2765	1950	4715	
Totals expressed as percentages			58·64	41·36	100	1·42
Bud	1	May 31	1794	2356	4150	
„	2	July 5	841	1007	1848	
„	3	July 25	420	340	760	
„	4	Aug. 22	380	260	640	
Totals for four cuts			3435	3963	7398	
Totals expressed as percentages			46·43	53·57	100	0·87
Early flower	1	June 22	1585	2699	4284	
„	2	July 25	993	1041	2034	
„	3	Aug. 29	615	552	1167	
Totals for three cuts			3193	4292	7485	
Totals expressed as percentages			42·66	57·34	100	0·74

Comments on Table III.

The results in the final column for the mean leaf-stem ratios over the whole season are perhaps the best index to the leafiness of the crop when cut systematically at any of the three stages of growth dealt with. The decrease in the ratio as the cutting is postponed to the later stages of growth is brought out prominently in every case.

The data show that the same variety of lucerne, when grown on different soils, may display differing leaf-stem ratios in one and the same season. Thus in 1933, with the variety Provence, the Willingham lucerne (two-year-old stand on heavy "skirt" land) gave higher ratios than the Howe Hill crop (one-year-old stand on a light gravelly soil). It may be observed that the greater leafiness of the Willingham lucerne was easily recognisable by eye.

It is also clear from Table III that the leaf-stem ratio of a lucerne crop may vary from season to season. Thus in 1934 the leaf-stem ratios of the Willingham crop were significantly lower than those for the crop in the previous season. It is remarkable how closely these 1934 values approximate to the 1933 ratios for the Howe Hill crop. The results under consideration, arrived at from investigations in two seasons only, are unfortunately too restricted to demonstrate any possible connection between the leafiness of the crop and the age of the stand.

In considering the yield data in Table III, it should be borne in mind that both 1933 and 1934 were characterised by drought. This had a marked effect on the yield of lucerne in the second half of the season. On the light soil at Howe Hill during 1933, no cuts were possible after July, although the effect was not so severe on the heavy soil at Willingham. Despite this, however, heavier total yields were obtained at Howe Hill, a result that must probably be ascribed to the warmer light soil promoting earlier growth and enabling the crop to take full advantage of the rains in the spring.

Table III has a special interest to such industrial concerns as are concerned with the manufacture of lucerne meal, since it furnishes information as to the yield and type of product obtainable under the three possible systems of cutting. The data should be considered in conjunction with previous findings as to the relative feeding values of lucerne when cut at the pre-budding, budding and flowering stages of growth (2). Of direct interest in this connection is the conclusion arrived at in a previous communication (3) as to the close agreement between lucerne-leaf meal, made from the foliage of a flowering crop, and lucerne meal made from the crop in its pre-budding stage of growth. Both these meals constitute a concentrate of moderate starch equivalent, furnishing a generous supply of digestible protein. They are replaceable, lb. for lb., in the rations of live-stock.

Whether lucerne should be utilised for the manufacture of lucerne-leaf meal or pre-budding lucerne meal must depend on two considerations: (1) the yield of product obtainable; (2) the influence of the system of cutting on the health and subsequent life of the stand. The data in Table IV furnish information on the first of these considerations. They suggest that, in the first year of the comparison at any rate, the yields of pre-budding lucerne meal should be rather higher than those of lucerne-leaf meal. They show also that the manufacture of the leaf meal from the flowering crop would introduce the complication of having to dispose of large amounts of dry fibrous material from the stem fraction of the crop.

Table IV. *Comparative yields of pre-budding lucerne meal and lucerne-leaf meal in terms of lb. dry matter per acre per season.*

	Lucerne meal from pre-budding crop lb. dry matter	Lucerne-leaf meal from flowering crop lb. dry matter	Lucerne-stem meal from flowering crop lb. dry matter
Howe Hill crop (1933)	4383	3433	4402
Willingham crop (1933)	3967	3547	4004
Willingham crop (1934)	4715	3193	4292

It should be pointed out, however, that in every case the yields of pre-budding lucerne shown in Table IV were determined on areas that had not in previous years been submitted to this severe system of cutting. The ultimate effect on yield of cutting, season by season, at this stage of growth is therefore not brought out. It may be assumed that continuous cutting at the flowering stage, for the purpose of manufacturing the leaf meal, would cause the minimum falling-off of productivity in subsequent seasons. On the other hand, however, it is generally recognised that continuous cutting at a stage before bud development reacts injuriously on the plant and materially shortens the life of the stand (2). Under such a system of cutting it is also difficult to prevent serious infestation of the crop by weeds and grasses.

It is probable, therefore, that a continuation of such treatment of the crop into the second and third seasons would lead to a much more rapid deterioration in productivity than when the crop is cut in early flower, and that the yields of pre-budding lucerne meal obtained in this way would soon compare disadvantageously with the yields of lucerne-leaf meal secured from the flowering crop. That this is the case is shown by the results of the present trials. In Table V are shown the yields from large-scale plots on the Willingham area that have been submitted to systematic cutting, through two seasons, at the pre-budding, budding and flowering stages of growth.

Table V. *Comparative yields of weed-free lucerne from plots submitted to systematic cutting at the pre-budding, budding and flowering stages of growth* (*Willingham*, 1933 1934).

	Pre-budding plot A lb. dry matter per acre	Budding plot B lb. dry matter per acre	Flowering plot C lb. dry matter per acre	Pre-budding plot D (cut as a flowering plot in 1933) lb. dry matter per acre
Season 1933:				
First cut	1788	3227	4125	—
Second cut	680	1968	2544	—
Third cut	976	1178	882	—
Fourth cut	385	—	—	—
Fifth cut	138	—	—	—
Totals for 1933	3967	6373	7551	—
Season 1934:				
First cut	998	4150	4284	1822
Second cut	459	1848	2034	1134
Third cut	397	760	1167	658
Fourth cut	150	640	—	579
Fifth cut	—	—	—	345
Sixth cut	—	—	—	177
Totals for 1934	2004	7398	7485	4715

It will be seen from Table V that during the first year of the experiment the yield from plot A, cut systematically at the pre-budding stage, was 52·5 per cent. of that of the flowering plot C. During the second year of such treatment, however, despite a spring dressing of superphosphate and muriate of potash, the productivity of plot A deteriorated considerably, the season's yield being only 2004 lb. of dry matter per acre, *i.e.* almost half of the previous year's yield and only 26·8 per cent. of the yield in the same season from the flowering plot. It was noteworthy that although the lucerne on the pre-budding plot A displayed quite a healthy growth in the spring of the second year of experiment, yet the plants in late April were 3 in. or more behind the growth on the budding and flowering plots. This effect was so marked that the boundary of the pre-budding plot was sharply delineated by this difference in height. That this marked deterioration in productivity is to be related to the frequent cutting of the plot in the previous season is evident from the fact that plot D, submitted for the first time in 1934 to systematic cutting at the pre-budding stage, gave a yield of 4715 lb. of dry matter per acre, *i.e.* nearly 2½ times the yield from plot A.

It may also be observed that the progressive increase in the weediness of plot A as the experiment proceeded was very apparent to the eye. Despite the use of the horse-hoe and harrow in the early part of 1934, the plot had taken on, by the end of the season, the appearance more of a grass plot than of a lucerne plot. Measurements made on May 31, 1934, for example, on samples from the pre-budding plot A showed a content of weeds as high as 40 per cent., compared with but 8 per cent. in samples from the budding plot B. In respect of weediness, therefore, the pre-budding plot stood out in marked contrast with the areas cut systematically at the later stages of growth. On these areas no disadvantageous influence on yield and plant vigour, attributable to the systems of cutting, could be noted.

It is possible that the deterioration of productivity brought about by cutting systematically at too early a stage of growth may have been exaggerated by the dry character of the seasons of experiment. The frequent cutting of the immature plant, with the consequent increased effect of restricting the size and extent of the root system, must render the crop less drought-resistant than when the plants are allowed a fuller development before cutting, a point that has been stressed by Nicol in a recent publication (4). The belief is justified, however, and is supported by ample evidence from American trials (5, 6), that a repetition of the experiments under weather conditions more favourable to growth of green crops would result in a similar deterioration of the frequently-cut lucerne, although this might not be displayed so promptly and on such a marked scale as in the present trials. The dwarfing of the roots from such a system of cutting would react adversely on the "photosynthetic" top growth. This decrease in top growth would prejudice still further the development of the root system and the maintenance of the root reserves on which prompt recovery after cutting largely depends. The continued interaction of these factors would lead to an inevitable running-off in the health and productivity of the lucerne and a consequent shortening of the life of the stand.

In order to throw further light on the influence of the different systems of cutting, as manifested in the two years of experiment, the crop *on all the plots* was permitted in 1935 to grow to the stage of *advanced bud* and the yields of *weed-free* lucerne determined on June 14. Plot C (cut systematically at the flowering stage in the previous two years) gave

4518 lb. of weed-free dry matter per acre, the green cut containing only 5·7 per cent. of weeds. Plot B (cut systematically at the budding stage in the previous two years) yielded 3956 lb. of dry matter per acre, the weeds in the green cut amounting to 10·3 per cent. Plot D, which in the previous year had been cut systematically at the pre-budding stage, gave only 2434 lb. of dry matter per acre, the weeds comprising 42 per cent. of the green cut, while on plot A, which had been submitted to systematic cutting at the pre-budding stage during the previous two seasons, the yield of weed-free dry matter fell to 2071 lb. per acre, weeds forming 42 per cent. of the green cut. The weeds were mainly grasses.

During the early part of the season, the pre-budding plots were easily picked out by the backward growth of the lucerne, while at the end of April the colourful effect of a profusion of flowering dandelions on the pre-budding areas caused these plots to stand out vividly from the rest of the field. These final data and observations emphasise the disadvantages of the frequent cutting of lucerne at an early stage of growth. It should be borne in mind that all the three winters of the experiment were unusually mild and open, and that the comparison might have been much more disadvantageous to the pre-budding plots had the weakened plants on these areas been subjected to the test of severe winter conditions.

THE SEPARATE COMPOSITION OF LEAF AND STEM.

The results of the analyses of the separate fractions of leaf and stem from the different cuts of pre-budding, budding and flowering lucerne at Howe Hill during the 1933 season are recorded in Table VI. Similar analytical work was carried out on the Willingham samples, but as these data presented no features that were not amply illustrated by the Howe Hill figures, it is not considered necessary to include them in this paper. For comparative purposes, the composition of a sample of actual flower is also shown in the table.

Comments on Table VI.

In all cases, at whatever stage of growth the sample was cut, the leaves are very conspicuously richer in protein and poorer in fibre than the stems. Even before the flower buds had appeared, the stems of the plant contained from about 31 to 37 per cent. of fibre (dry matter basis), whereas the leaves contained no more than 11–13 per cent. This finding throws light on the conclusion drawn from the results of an earlier investigation, that one of the main nutritional characteristics of the lucerne

Table VI. *Separate composition of leaf, stem and flower from Howe Hill lucerne, season* 1933 (*on basis of dry matter*).

(1) *Pre-budding cuts.*

No. of cut ...	1		2		3		4	
	Leaf %	Stem %	Leaf %	Stem %	Leaf %	Stem %	Leaf %	Stem %
Crude protein	30·13	16·97	34·80	19·12	30·70	14·11	30·63	13·31
Ether extract	3·43	1·20	3·19	1·05	4·06	1·52	4·49	1·80
N-free extractives	41·19	41·28	37·60	32·14	41·70	35·87	40·39	36·56
Crude fibre	12·99	30·77	11·60	32·27	11·16	36·98	11·79	36·72
Ash	12·26	9·78	12·81	15·42	12·38	11·52	12·70	11·61
SiO$_2$-free ash	12·12	9·62	11·73	14·88	12·06	11·29	12·55	11·52
Lime (CaO)	4·16	1·71	3·18	1·99	4·09	2·17	4·05	2·23
Phosphoric acid (P$_2$O$_5$)	0·96	0·74	1·16	0·94	1·00	0·70	0·98	0·68
Potash (K$_2$O)	2·69	3·99	3·57	6·91	2·52	4·42	2·81	4·49
Soda (Na$_2$O)	0·40	0·29	0·01	0·03	0·47	0·40	0·07	0·11
Chlorine (Cl)	0·36	0·52	0·30	0·82	0·38	0·77	0·45	0·79
True protein	25·01	10·22	28·45	10·30	25·90	9·24	25·49	9·45
"Amides"	5·12	6·75	6·35	8·82	4·80	4·87	5·14	3·86

(2) *Budding cuts.*

No. of cut ...	1		2		3	
	Leaf %	Stem %	Leaf %	Stem %	Leaf %	Stem %
Crude protein	27·45	12·48	29·70	15·19	28·14	10·55
Ether extract	3·01	1·15	4·24	1·69	4·96	1·50
N-free extractives	43·53	36·14	40·78	33·69	41·85	34·74
Crude fibre	12·33	42·46	12·19	39·07	11·56	44·16
Ash	13·68	7·77	13·09	10·36	13·49	9·05
SiO$_2$-free ash	13·60	7·68	12·74	10·15	13·40	8·97
Lime	5·36	1·91	4·60	1·93	4·70	1·69
Phosphoric acid	0·76	0·54	0·98	0·77	0·84	0·63
Potash	1·28	2·18	2·35	4·02	2·86	3·64
Soda	0·24	0·77	0·28	0·19	0·14	0·22
Chlorine	0·43	0·46	0·35	0·71	0·37	0·36
True protein	22·09	7·69	25·59	8·37	23·97	7·42
"Amides"	5·36	4·79	4·11	6·82	4·17	3·13

(3) *Flowering cuts and sample of flower.*

No. of cut ...	1		2		
	Leaf %	Stem %	Leaf %	Stem %	Sample of flower %
Crude protein	23·48	9·44	26·33	10·32	22·22
Ether extract	3·01	1·03	4·04	1·58	2·04
N-free extractives	46·18	38·14	43·21	35·18	52·86
Crude fibre	13·39	44·36	13·21	45·61	16·29
Ash	13·94	7·03	13·21	7·31	6·59
SiO$_2$-free ash	13·82	6·94	13·11	7·22	6·45
Lime	5·59	1·79	4·95	1·59	1·46
Phosphoric acid	0·63	0·47	0·70	0·62	1·00
Potash	2·14	2·32	1·87	2·67	2·30
Soda	0·07	0·04	0·22	0·01	0·17
Chlorine	0·50	0·36	0·58	0·40	0·28
True protein	19·22	6·22	21·25	6·85	13·69
"Amides"	4·26	3·22	5·08	3·47	8·53

crop is "the readiness with which the young plant produces fibre, and the early stage of growth at which the fibre begins to display signs of lignification and diminished digestibility. The fact that the protein and N-free extractives show no correspondingly early running-off in digestibility suggests that this development and lignification of fibre is taking place mainly in the stems, and not to any significant extent in the leaves of the plant. It is obviously to these processes that lucerne owes its habit of erect growth (2)."

In keeping with this statement is the proof afforded by both the Howe Hill and the Willingham data that, throughout the whole period of growth of the plant, the leaves display a much higher degree of uniformity in protein and fibre content than the stems. The essential data illustrating this feature are brought together in Table VII. They refer to the first cuts taken at the three different stages of growth.

Table VII. *Protein and fibre content of leaf and stem at the pre-budding, budding and early-flowering stages of the lucerne plant (dry-matter basis).*

	Howe Hill (1933)				Willingham (1933)			
	Leaf		Stem		Leaf		Stem	
First cut at:	Crude protein %	Crude fibre %	Crude protein %	Crude fibre %	Crude protein %	Crude fibre %	Crude protein %	Crude fibre %
Pre-budding stage	30·13	12·99	16·97	30·77	32·93	12·47	19·46	30·94
Budding stage	27·45	12·33	12·48	42·46	28·38	13·19	13·57	42·88
Early-flowering stage	23·48	13·39	9·44	44·36	24·57	13·94	11·03	46·49

It will be noted from Table VII that the fibre content of the leaves undergoes very little change during the growth of the crop, whereas the stems become more and more fibrous. Nearly half the dry matter of the stems in the stage of flower consists of fibre. Clearly the increase in fibre which takes place during the growth of lucerne is not to be related to a change in the leaves but to an increase in both the amount of stems and the percentage of fibre that they contain. In respect of protein content also, the fall in the case of the leaves is much more restricted than in the stems. If the Howe Hill data be considered from this standpoint, it is seen that the range of fall in stem protein represented 44 per cent. of the crude protein originally present, whereas the corresponding figure for leaf protein was only about 22 per cent.

It is also to be noted from Table VI that the leaves at all stages of growth are very definitely richer in ether extract than the stems. It has already been shown (3) that hay-making results in a significant lowering of the ether extract in lucerne, and evidence has been adduced to prove that this depression is brought about by the exposure of the forage to sunlight in the field-drying stage. The data of the present investigation suggest that the breaking-off of leafage, which is difficult to avoid when converting lucerne into hay, may contribute seriously to this diminution in the percentage of ether extract. The obvious means of preventing this loss is to collect the forage soon after cutting and to dry it by artificial means.

The stems are also somewhat poorer in N-free extractives than the leaves, but the difference in this respect is not so pronounced as with the fibre and protein. Only in the case of the first cut of pre-budding lucerne is this distinction not to be detected. In respect of mineral constituents, the difference in composition between the leaves and stems again becomes pronounced, the leaves in general displaying the higher ash content. This distinction is very much more pronounced in the stages of bud and flower than in the earliest stage of growth, a characteristic that was also shared by the Willingham lucerne, in which case, at the flowering stage, the leaves (14·4 per cent. of SiO_2-free ash on the basis of dry matter) were more than twice as rich as the stems (5·2 per cent.). The ash content of the leaves apparently undergoes little change with advancing growth of the crop, while that of the stems, on the other hand, suffers considerable reduction.

The second pre-budding cut of Howe Hill lucerne stands out on account of a seemingly abnormal feature, in that the stems of this cut were actually richer in SiO_2-free ash than the leaves, a result to be ascribed to the sudden and somewhat unaccountable rise in the potash content of the stems. The Willingham crop displayed the same behaviour, the second pre-budding cut differing from all the other cuts, irrespective of stage of growth, in having a higher content of SiO_2-free ash in the stems (10·0 per cent.) than in the leaves (9·4 per cent.). The exceptional character of this cut is not to be attributed to an influence of the weather since the disturbing effects of drought had not come into play at the date of taking the sample. It has been shown in a previous publication (2) that the second cut of pre-budding growth both at Howe Hill and Willingham (1933) was in other respects different from the other cuts. It displayed the lowest content of dry matter, the highest content of crude protein and "amides", the lowest content of fibre and the highest content of phosphoric acid; further, its leaf-stem ratio was higher than that of either the first or third cut. The conclusion has been drawn that this growth represents a more immature phase of development than either the first or third pre-budding growth, and that this circumstance is to be held responsible for its unusual characteristics (2).

In all the cuts at all stages of growth, the leaves are very much richer in lime than the stems. This emphasises again the necessity for making every effort to avoid the breaking-off of the brittle foliage when making lucerne hay. The leaves are also richer in phosphoric acid, but the distinction between leaf and stem is not so pronounced in this respect as in the case of the lime constituent. In both leaf and stem the percentage of phosphoric acid tends to run off with increasing maturity of the cuts, a result in harmony with an earlier finding that the percentage of phosphoric acid in the whole plant decreases as the crop progresses in maturity (1). The lime content of the whole plant has been shown to display the opposite variation, although the range of this variation appears to differ with different crops of lucerne (1). The increasing lime percentage would appear, from the data in Table VI, to be associated with changes in the leaves rather than the stems, an inference that is also borne out by the composition of the Willingham (1933) lucerne, where, on the basis of dry matter, the percentages of lime in the leaves of the first cuts at the pre-budding, budding and flowering stages were 4·26, 4·82 and 6·89 respectively, the corresponding values for the stems being 2·13, 1·63 and 1·82 per cent.

In respect of potash, the leaves in the pre-budding cuts are in every case decidedly poorer than the stems. Attention has already been called to the very high percentage of this constituent in the stems of the second cut. Both leaves and stems show a falling-off in potash content with increasing maturity, although the stems continue still to be richer than the leaves. A similar decrease in potash has been shown to occur when the mineral changes in the plant as a whole are investigated (1).

The stems of the pre-budding cuts are significantly richer in chlorine than the leaves, but with the further growth of the crop the position becomes reversed, due to a slight rise in the chlorine content of the leaves and a somewhat more pronounced fall in the stems. This behaviour was shared by the Willingham crop, the chlorine content of the leaves, on the dry-matter basis, rising from 0·23 per cent. in the first pre-budding cut to 0·29 per cent. in the first flowering cut, while the corresponding change in the stems was from 0·41 to 0·21 per cent.

The composition of the leaf sample from the first cut of flowering lucerne (see Table VI) should give some criterion of the quality to be aimed at in the manufacture of lucerne-leaf meal from a flowering crop. It is of interest therefore to compare these data with those for the sample of American lucerne-leaf meal dealt with in an earlier stage of these experiments (3). The comparison is set out in Table VIII and is restricted to a consideration of the organic constituents, since previous work has shown that climate, soil conditions and manurial factors may exert a pronounced influence on the mineral composition of lucerne.

Table VIII. *Composition of leaf sample from first cut of lucerne in flower (Howe Hill, 1933) compared with that of American lucerne-leaf meal (dry-matter basis).*

	Leaf from lucerne in early flower %	American lucerne- (3) leaf meal %
Crude protein	23·48	23·53
Ether extract	3·01	2·06
N-free extractives	46·18	44·62
Crude fibre	13·39	17·59
SiO_2-free ash	13·82	10·32

It will be noted from Table VIII that the agreement in content of protein and N-free extractives between the lucerne-leaf meal and the sample of leaf from lucerne in flower is quite satisfactory. The leaf meal, however, is more fibrous and rather poorer in ether extract and ash. It was made by grinding and sifting lucerne hay, and would appear to contain a somewhat higher proportion of stem than was present in the leaf sample with which it is compared in Table VIII.

It remains finally to consider the data in Table VI for the composition of lucerne flowers. In respect of protein and fibre, the flowers clearly exhibit a much closer agreement with the leaves than the stems. The "amide" content of the flowers is, however, much higher than that of both the leaves and the stems. The percentages of ash and lime in the flowers are very much lower than those in the leaves, and in these respects there is very close correspondence between the flowers and the stems. The phosphoric acid content is somewhat higher, and the chlorine content somewhat lower, than in both stems and leaves. Broadly speaking, it may be concluded that the flowers tend to follow the leaves in organic composition and the stems in respect of mineral content.

INFLUENCE OF VARIETY ON THE LEAF-STEM RATIO.

With the collaboration of the National Institute of Agricultural Botany, Cambridge, it was possible to make determinations of the leaf-stem ratios of the first two cuts from a number of small rectangular plots, lying side by side on a heavy clay soil, on which a series of varieties and strains of lucerne were growing. The plots were 11 ft. long and contained four rows of lucerne, the distance between the rows being 1 ft. The scything of the plots was always done on fine mornings when the forage was carrying no superficial moisture, so that the figures in the yield column of Table IX represent the weight of air-dry crop from the plots as determined immediately after cutting. The year of sowing in every case was 1931.

Table IX. *Summary of leaf-stem ratios as determined on different varieties of lucerne (season* 1933).

No. of cut	Date (1933)	Variety	Air-dry yield of green crop lb. per plot	Stage of growth	% dry matter in Leaf	% dry matter in Stem	Leaf dry matter as % of total dry matter	Stem dry matter as % of total dry matter	Leaf-stem ratio
1	May 29	English*	60	Advanced bud	23·0	22·2	48·59	51·41	0·95
2	July 26		47	¾ flower	26·6	32·7	51·44	48·56	1·06
1	May 29	Grimm	51	Advanced bud	22·8	20·7	49·46	50·54	0·98
2	July 26	(Garton's)	56	¾ flower	26·7	32·0	47·76	52·24	0·91
1	May 29	Argentine	45	Advanced bud	22·7	20·7	48·24	51·76	0·93
2	July 26		56	½ flower	26·3	32·3	46·92	53·08	0·88
1	May 29	Grimm	54	Advanced bud	23·0	21·9	47·75	52·25	0·91
2	July 26	(direct)	63	¾ flower	26·1	32·1	48·80	51·20	0·95
1	May 29	Provence	46	Advanced bud	23·0	22·9	47·75	52·25	0·91
2	July 26		46	¾ flower	26·9	33·8	51·56	48·44	1·06
1	May 29	"Medanos"	35	Early bud	22·2	20·4	50·19	49·81	1·01
2	July 26	(Italian)	51	½ flower	26·5	31·3	42·90	57·10	0·75
1	June 15	Provence	46	Early flower	27·6	31·6	48·29	51·71	0·93
2	July 26		42	½ flower	25·5	29·8	52·38	47·62	1·10
1	June 15	Hungarian	44	Early flower	28·9	33·7	46·62	53·38	0·87
2	July 26		46	½ flower	26·7	29·5	50·79	49·21	1·03
1	June 15	Fekete	52	Early flower	29·0	33·5	46·40	53·60	0·87
2	July 26	(Hungarian)	42	½ flower	26·0	29·9	51·31	48·69	1·05

* The term "English" denotes lucerne grown from seed originating from Provence lucerne that has been grown in this country for 2 or 3 years.

Comments on Table IX.

It is not desirable to stress the comparative data in Table IX unduly, since the information to be derived from these figures can have no absolute value. The performance of any given variety must in some measure be dependent on the conditions of soil and climate under which it is tested. A variety showing satisfactory yield and leafiness under the present conditions of experiment might conceivably not display an equally good behaviour on a different type of soil or in a region of widely different climate. Any conclusions based on the data in Table IX, therefore, refer to the relative merits of the different varieties under the conditions of soil and climate incidental to this particular trial.

It is of interest to note, however, that the several varieties did not show any very serious differences in respect of leaf-stem ratios. This was particularly true of the first cut of the season. If the results for this cut, taken in advanced bud, be compared for the first five plots (including English, two plots of Grimm and one each of Provence and Argentine), it will be seen that the range of variation was merely from 0·91 to 0·98. In the case of the second cut in flower, Provence and English displayed a somewhat higher degree of leafiness than Grimm and Argentine, but this advantage was rather offset by the lower yields from the former.

The Italian "Medanos" compared disadvantageously with the other varieties. It made relatively slow progress during the first part of the season, and at the time of the first cut the plants were smaller than on the other plots and budding was less advanced. The yield of green crop was comparatively poor, although the leaf-stem ratio compared quite satisfactorily with the values for the first cuts of the other varieties. The "Medanos" grew more satisfactorily for the second cut, but the leaf-stem ratio in this case was the lowest of all the values recorded in Table IX.

The results for the three remaining plots (Provence, Hungarian and Hungarian Fekete) call for no special comment. It merely remains to point out that, leaving out the figures for the Italian variety, the results in general again demonstrate how the later cuts tend to display a greater degree of leafiness than the first cut of the season.

Analyses were made of the cuts from the different varieties of lucerne, as well as of the separate leaf and stem fractions, but as the data presented no new features and gave no indication of significant distinctions that might be attributed definitely to differences of variety, it is considered unnecessary to record the figures in this paper.

SUMMARY.

In this paper are brought together a number of data concerning the ratio of the amount of leaf to stem in the lucerne crop, at different stages of growth, and the separate composition of the leafy and stemmy fractions.

It is shown that the proportion of leaf falls off as the crop advances in maturity, and that the rate of decrease of leafiness is greater before budding than during the period from the beginning of budding to early flower. The changes in the leaf-stem ratio largely account for the fall in protein content, the rise in fibre content and the running-off in digestibility and feeding value that take place as the crop passes through the successive stages of growth up to flowering.

The values of the leaf-stem ratios show that the later cuts in the season have a distinctly leafier character than the early cuts. This is the case whether the cuts are made systematically at the pre-budding, budding or flowering stage of growth and accounts for the observed higher protein content of the late-seasonal cuts. To delay too long before taking the first cut of lucerne involves the danger of a greater reduction in the percentage of leaf in the crop, and consequently in the feeding value of the hay, than would be caused by a similar delay in the later part of the season.

The problem of the conservation of lucerne by artificial drying is discussed on the basis of the present results. Whether the crop should be utilised for the manufacture of lucerne-*leaf* meal from flowering lucerne or lucerne meal from pre-budding lucerne (two products that have been shown in a previous investigation to be of almost equal concentrate value) depends on two considerations: (1) the yield of product obtainable; (2) the influence of the system of cutting on the health and life of the stand. The present results suggest that the yields of pre-budding lucerne meal would be rather higher than that of the lucerne-leaf meal in the first year of cutting. A continuation of the comparison into the second season, however, showed that the lucerne cut systematically in the pre-budding stage displayed a very serious deterioration in productivity and also compared very disadvantageously in respect of weediness with the lucerne cut less frequently at a more advanced stage of growth. Attempts to secure a lucerne meal of concentrate value by frequent cutting of the crop at a very early stage of growth are therefore not to be encouraged. The manufacture of such a meal should take the form of making lucerne-leaf meal from the foliage of the flowering crop.

Separate analyses of the leaf and stem fractions of the lucerne cuts showed that at all stages of growth the leaves are conspicuously richer in protein and poorer in fibre than the stems. Even before the flower buds had appeared, the stems contained from 31 to 37 per cent. of fibre (dry-matter basis), while the leaves contained no more than 11–13 per cent. Nearly half the dry matter of the stems in the stage of flower consists of fibre. The leaves display, throughout the whole period of growth, a much higher degree of uniformity in protein and fibre content than the stems. They are also very definitely richer in ether extract and slightly richer in N-free extractives. The difference in composition between leaf and stem again becomes pronounced in respect of mineral constituents, the leaves, particularly in the stages of bud and flower, showing the higher ash content. The amount of ash in the leaves undergoes little change with advancing growth of the crop, while that of the stems suffers considerable reduction.

In all the cuts, at all stages of growth, the leaves are very much richer in lime than the stems. They are also richer in phosphoric acid, but the difference in this respect is not so pronounced as in the case of the lime. The leaves of the immature cuts are in every case decidedly poorer in potash than the stems. Both leaves and stems show a falling-off in potash content with increasing maturity, although the stems still continue to be richer than the leaves. The stems of the pre-budding cuts are also richer in chlorine than the leaves, but the position becomes reversed with the further growth of the crop. The flowers of lucerne tend to follow the leaves in organic composition and the stems in respect of mineral content.

In the final section of the paper are recorded the results of the determination of the leaf-stem ratios of the first two cuts from a number of varieties of lucerne growing side by side on a heavy clay soil at Cambridge.

The writers, in conclusion, take this opportunity of expressing their indebtedness to their colleague, Mr A. Eden, B.A., for valuable assistance in the foregoing investigation. Thanks are also due to Mr S. F. Armstrong, M.A., of the National Institute of Agricultural Botany, for help in the investigation of the leafiness of lucerne in relation to variety.

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(*Received June 24th*, 1935.)

AN EXAMINATION OF METHODS FOR DETERMINING ORGANIC CARBON AND NITROGEN IN SOILS[1].

By ALLAN WALKLEY.

(Chemistry Department, Rothamsted Experimental Station, Harpenden, and the Waite Agricultural Research Institute, Adelaide.)

(With One Text-figure.)

As a preliminary to the examination of a large series of soil samples from the plots of the Woburn Experimental Station, a critical study was made of a number of the current methods for determining organic carbon and nitrogen in soils. Special attention was given to relatively simple and rapid methods which are suitable for examining large numbers of closely comparable soils even though they give incomplete recoveries and need empirical standardisation. The modified Kjeldahl method of Robinson, McLean and Rice Williams (1) (subsequently referred to as the Bangor method) was finally adopted for the Woburn soils because carbon and nitrogen could conveniently be determined on the same soil sample. A modification of Schollenberger's rapid chromic acid titration method (2) which was developed in conjunction with Armstrong Black and briefly described elsewhere (3), was re-examined and the technique improved to give greater convenience and to overcome the errors due to the large amounts of chlorides in certain Australian soils.

THE DENNSTEDT DRY COMBUSTION METHOD FOR CARBON.

This method (4) does not appear to have been widely used by British soil chemists, though it is well known on the Continent and has several advantages over the more usual dry combustion method originally due to Liebig. The combustion is conducted in oxygen which enters the combustion tube in two separate streams through concentric tubes, a long inner one, which is widened to admit the boat containing the sample, and a short outer one. The stream of oxygen passing through the inner tube and over the boat, sweeps along any unburnt gases and unites with the stream of oxygen from the shorter inlet tube. The streams meet near strips of platinum foil arranged to give a star-shaped cross-section and heated to low redness. Efficient oxidation on the platinum surface is an essential feature of the Dennstedt method. The combustion proceeds at a relatively low temperature, three ordinary burners replacing the usual array of burners or the powerful electrical heating units of the Liebig furnaces.

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[1] This paper is based on a portion of a "Thesis approved for the Degree of Doctor of Philosophy in the University of London", and some later work in Adelaide.

The only filling in the combustion tube is lead peroxide, which is held at about 320° C. and removes oxides of nitrogen and the halogens. Dennstedt advised the use of a mixture of lead peroxide and red lead, though Pregl (5) used lead peroxide alone. This seems adequate, for some red lead is formed during the combustion. After considerable difficulty in obtaining lead peroxide free from organic matter, Merck's "special lead peroxide for Pregl method" was found to be satisfactory. Instead of using the lead peroxide in a boat open at one end, as recommended by Dennstedt, 30–50 gm. in the form of small granules were packed into a cylinder of copper gauze which filled the combustion tube. Dennstedt recommended that after removing the moisture by calcium chloride the carbon dioxide should be absorbed in a large soda-lime tube followed by a smaller one containing soda-lime and calcium chloride in separate compartments. In this work the large tube was found to be clumsy and was omitted. The smaller tube was filled with "sofnolite" and calcium chloride, and gave full absorption with a gas stream of 1–2 litres per hour. From the absorption tubes the gases passed through a wash-bottle containing a dilute solution of palladious chloride in which a black precipitate is formed by traces of carbon monoxide in the event of incomplete combustion.

With a 10 × 1 cm. boat and 100 mesh (0·13 mm.) soil yielding 0·1–0·2 gm. carbon dioxide the time for combustion was about 1 hour, but this might have been reduced by using a larger absorption tube. The analyses presented in Table III were made by the technique so far described. Some possible simplifications were subsequently investigated.

Owing to its dilution in a great body of inorganic material, the soil organic matter burns away quietly, and the operation does not require such careful attention as is needed for volatile or rapidly decomposing organic compounds. It seemed probable therefore that the apparatus might be simplified by omitting several refinements. The platinum star frequently glows brightly during the combustion of pure organic compounds, but it did not do so for soils, presumably because most of the oxidation proceeded at the surface of ferric oxide and other active inorganic catalysts in the soil itself. A simplified apparatus with a single oxygen inlet and without a platinum star gave satisfactory results for several mineral soils. For a peat soil the result was about 2 per cent. too low and a trace of carbon monoxide was detected. This simplified technique has not been fully tested, but it is unlikely that any mineral soils would offer more difficulties than the peat.

On combustion amino- and imino-compounds liberate practically all of their nitrogen as gas, while substances with nitro- and azo-groups yield higher oxides of nitrogen (Pregl (5), p. 28). It was found that about 15 per cent. of the soil nitrogen was recovered as nitrate in the lead peroxide filling, and since the amount of nitrogen in soil is commonly about one-tenth of the carbon, the error by neglecting the oxides of nitrogen would be only a small percentage of the amount of carbon. Analyses without lead peroxide filling confirmed the relative unimportance of this source of error. It is probable that the lead peroxide could be omitted in the combustion of many soils, but it is necessary to retain it where sulphurous acid has been used to remove carbonates and for soils containing sulphides or chlorides. Probably the most effective filling in a single-inlet combustion tube without a platinum star would be lead peroxide with some manganese dioxide.

THE BANGOR METHOD FOR CARBON AND NITROGEN.

The technique for the Bangor method of determining soil carbon by measuring the amount of the sulphur dioxide liberated in a Kjeldahl digestion was carefully standardised. The correction factors for carbon and nitrogen were found to vary with the temperature of digestion. In the original description of the method (1) it was stated that the liquid must boil vigorously, but considerable superheating is possible. In experiments in which the temperature of the boiling liquid was measured it was found that the percentage recovery of carbon from a Woburn soil increased from 84 per cent. at 337° C. to 92 per cent. at 355° C. For the present work and for the examination of the Woburn soils the conditions were closely standardised by giving a preliminary gentle heating for 30 min., until the contents of the flask reached the final colour, and then digesting for an hour with sufficient heating to cause the liquid to reflux in the neck of the flask. The recovery varied with the type of soil, but was reasonably constant and closely reproducible for similar soils analysed at different times. Thus, with the standardised heating the mean differences in percentage recoveries between duplicates performed at intervals of a week were 1·50 per cent. for carbon and 1·65 per cent. for nitrogen on a group of twenty-six Woburn barley soils, as compared with 2·68 per cent. and 3·26 per cent. respectively for nineteen of the same soils without standardised heating. For seven Woburn soils the recovery of carbon with standardised heating varied between 92 and 95 per cent., and for fifteen soils from a rotation experiment in the Sudan Gezira between 81 and 87 per cent.

Two possible sources of error in grinding soils to pass the 100 mesh (0·13 mm.) sieve should be noted. A series of abnormally high results for some sandy soils by the Bangor method was traced to the presence of small particles of metal produced by abrasion during grinding in an iron end-runner mill. A trace of metallic iron will greatly increase the reducing power and apparent carbon content of a sandy soil in any method involving the reduction of an acid solution. Grinding by hand in a porcelain mortar was compared with grinding in a porcelain ball mill. Almost all clay soils examined gave similar results by both methods, but, contrary to expectation, sandy soils, which had lost their characteristically gritty feel by ball-mill grinding, gave lower results than by hand grinding. The apparent loss of organic matter proved to be due to dilution of the soil by material abraded from the mill. This was confirmed by the loss in weight of the mill.

The above experiments showed that extremely fine grinding was not essential for the Bangor method. In most soils good results were obtained with material ground to pass only a 1 mm. sieve; finer grinding is, however, often necessary for adequate sampling.

THE DETERMINATION OF NITROGEN IN HEAVY CLAY SOILS.

A heavy alkaline soil from the Sudan Gezira gave considerable increases in both carbon and nitrogen by the Bangor method as the result of fine grinding. In 1925 D. V. Bal (6) showed that the Kjeldahl method gave considerably higher nitrogen contents in certain heavy Indian cotton soils when water was added before the sulphuric acid. At about the same time and independently, H. J. Page and the late G. C. Sawyer at Rothamsted observed the same effect for this Gezira soil. Recently other examples have been given (7, 8). Bal suggested that the low results by the ordinary Kjeldahl procedure were due to the presence of some cementing substance soluble in dilute but not in concentrated sulphuric acid. The final residues are dark crumbs when concentrated acid is used directly, but after previous treatment with dilute acid, the final residues are finely divided and white. Sieving the Sudan soil to remove the nodules of calcium carbonate and manganese dioxide or previous leaching with

N/50 hydrochloric acid, followed by drying, had no effect on the recovery of nitrogen by treatment with concentrated acid. Very fine grinding without addition of water gave higher results than adding water before digesting the 1 mm. sample (Table I). In order to ascertain whether this effect was general for heavy soils, a number of samples of widely differing types were digested for 1½ hours with 25 ml. concentrated sulphuric acid for the combined carbon and nitrogen determinations. Similar determinations were made, for nitrogen only, after previously allowing the soils to stand with 25 ml. of water for 15 min. (The addition of water interferes with carbon determinations by the Bangor method.) From the data in Table II it will be seen that only two soils, from Southern Rhodesia and the Sudan, showed marked differences between the "wet" and "dry" methods. Both of these soils were strongly alkaline. Since fine grinding overcomes the difficulty, it would appear that the error does not depend on the production of insoluble cements such as iron and aluminous sulphates, but on the failure of the crumbs in certain heavy alkaline soils to disperse in the non-polar sulphuric acid. For the Kjeldahl method the addition of water is not necessary for many heavy soils, but it is preferable, if only because the digestion proceeds more smoothly and with less

Table I. *Effect of fine grinding and water on nitrogen and carbon determinations in Gezira soil.*

	% nitrogen Kjeldahl method	Bangor method
1 mm. soil: Without water	0·0204	0·170
With water	0·0249	
Ground in ball mill: Without water	0·0274	0·289
With water	0·0281	

Table II. *Carbon and nitrogen determinations in heavy soils.*

Soil	Clay %	*p*H	% recovery of carbon by Bangor method	% nitrogen by Kjeldahl method: "wet" *a*	"dry" *b*	$\frac{a-b}{a}.100$
Gloucester	41	7·1	84	0·548	0·542	1
Purleigh	32	7·0	96	0·233	0·234	0
Rothamsted	30	5·8	94	0·183	0·184	0
Cockle Park	30	5·6	93	0·150	0·155	−3
Rothamsted subsoil	—	4·2	—	0·101	0·102	−1
Mauritius	75	6·8	77	0·199	0·192	3
Southern Rhodesia (heavy vlei)	—	8·3	59	0·100	0·081	19
[illegible]	42	4·0	—	0·092	0·092	0
Southern Rhodesia (red soil)	—	6·6	77	0·064	0·065	−2
Tanganyika (lateritic clay)	—	5·2	76	0·054	0·053	2
Sudan Gezira	60	9·4	50	0·025	0·020	20

bumping. For heavy alkaline soils low in organic matter, it is advisable to grind the soil very finely, and also to add water. For the Bangor method it is essential that such soils should be ground very finely.

THE HYPOBROMITE METHOD FOR CARBON.

Sodium hypochlorite was used by Bornträger (9) over thirty years ago for determining soil humus by titrating an alkaline extract until it became colourless. A known amount of Kasseler Brown was similarly treated and used as a basis of comparison. For rapid field survey work Lapicque and Barbé (10) added hypochlorite directly to the soil and determined the excess iodometrically. Troell (11) showed that sodium hypobromite was effective in removing organic matter from soils. This reagent has the advantage that it is readily prepared by adding bromine to sodium hydroxide solution.

Experiments on the determination of soil organic carbon were made by shaking soil with hypobromite and allowing to stand overnight. Parallel experiments were conducted with Merck's Acidum Huminicum and, later, with a soil of known carbon content as standard. An aliquot of the clear supernatant liquid was removed, treated with potassium iodide and hydrochloric acid and titrated with sodium thiosulphate in the presence of starch. Preliminary experiments showed that a considerable excess of sodium hypobromite was required and the solution finally adopted was prepared by adding 2·5 ml. bromine to 150 ml. M NaOH (giving 2 parts $M/2$ NaOBr to 1 part M NaOH). The change of hypobromite to bromate, thoroughly studied by Skrabal (12), does not interfere with the titration, which depends on the total oxidising power of the solution. Titrations of the hypobromite alone by means of arsenious acid showed that in the above solutions only about 3 per cent. of the iodine titration value was due to bromate, and also that this amount was not increased in the presence of soil.

5 gm. of soil passing the 1 mm. sieve were treated with 100 ml. of the above solution, shaken a few times at intervals over 2 hours, left overnight and reshaken. When the soil had settled, 10 ml. of liquid were pipetted off, 10 ml. of 20 per cent. potassium iodide solution and 2 ml. of $6N$ hydrochloric acid added and the iodine titrated with $N/2$ thiosulphate. The results showed relatively poor recoveries, and the method was abandoned in favour of the dichromate method discussed below. Springer (13) has suggested that the amount of decomposition of hypochlorite by unit soil organic matter might be used to classify soils according to the ease of oxidation of their organic matter.

THE DICHROMATE METHOD.

This modification of Schollenberger's rapid method (2) of determining organic carbon by oxidising it with chromic and sulphuric acids at 175° C. and titrating the residual chromic acid arose out of the examination of a proposed modification by Degtjareff (14). As already described by Walkley and Armstrong Black (3), the method consists in treating an amount of soil containing 10–25 mg. carbon and passing the 100 mesh (0·13 mm. sieve) with 10 ml. of *N* $K_2Cr_2O_7$ in a 350 ml. conical flask followed by 20 ml. of strong sulphuric acid from a quick-delivery automatic pipette. The heat evolved is sufficient to raise the temperature to about 120° C. The mixture is shaken for 1 min., cooled under the tap and diluted to about 150 ml. with water. 5 gm. of sodium fluoride are added, and as soon as this has dissolved the excess dichromate is titrated with 0·4*N* ferrous ammonium sulphate, using 1 ml. of 0·5 per cent. diphenylamine as indicator. If the end-point is overshot the mixture can be back-titrated with potassium dichromate solution used as oxidant. If more than 75 per cent. of the dichromate is reduced, the determination should be repeated with less soil.

The method is extremely rapid, taking only 10–15 mins., and should be useful in survey work where no great accuracy is required.

COMPARISON OF THE DENNSTEDT, BANGOR, DICHROMATE AND HYPOBROMITE METHODS FOR ORGANIC CARBON.

Table III gives the results of comparisons of the Dennstedt, Bangor, dichromate and hypobromite methods for a series of British and foreign soils. Eight of the soils were used in a co-operative study of the Bangor method by a Committee of the Agricultural Education Association, whose report (15) should be consulted for further details and recommendations. Some of the analyses in Table III were for single samples. The dry combustion method is the only one of the four which is capable of giving accurate results over a variety of soils. The variabilities of the recovery factors for the three other methods are shown by expressing the standard error per determination as a percentage of the mean percentage recovery. It will be seen that the hypobromite method gave much more irregular results than the Bangor or dichromate methods. The dichromate method was only slightly inferior to the Bangor method. It is clear that neither of these methods could be used for such a wide variety of soils as those in Table III, unless the appropriate recovery factors were determined for the main types of soil. Both methods may, however, be used satisfactorily for groups of comparable soils with occasional determinations of the recovery factor. The Bangor method has the advantage of determining both carbon and nitrogen in the same sample, but the dichromate method is much cheaper and more rapid.

Table III. *Comparison of four methods for determining organic carbon in soils.*

Origin of soil	Description	% organic carbon by combustion	% recovery by Bangor method		
			Bangor method	$K_2Cr_2O_7$ method	NaOBr method
Great Britain:					
Arnish Moor*	Peat	45·8	101	86	—
Gloucester	Clay loam	5·43	84	74	62
Insch*	Loam	4·88	100	83	62
Craibstone*	Sandy loam	3·88	100	80	63
Bangor*	Clay loam	3·06	97	76	64
Purleigh	Clay loam	2·29	96	81	74
Cockle Park*	Clay	1·98	93	78	57
Rothamsted*	Clay loam	1·87	94	79	68
Harper Adams*	Light sand	0·91	99	72	—
Woburn*	Sandy loam	0·87	93	71	56
Abroad:					
U.S.S.R.	Chernozem	5·80	91	80	63
Swaziland	Heavy black soil	2·84	86	68	—
Mauritius	Heavy clay	2·18	77	78	71
Southern Rhodesia	Heavy black vlei	1·45	88	74	68
Malay	Clay loam	1·24	92	78	67
Swaziland	Red soil	1·19	89	76	—
Tanganyika	Heavy lateritic clay	0·63	76	75	60
South Australia	Sandy loam	0·40	90	74	50
Sudan Gezira	Heavy alkaline clay	0·38	85	60	—
Mean percentage recovery			90·4	75·8	64·7
Standard error per determination			7·7	5·6	8·3
Standard error per determination as percentage of mean recovery			8·6	7·4	12·8

* Soils used in co-operative work of Agricultural Education Association.

MODIFICATION OF THE DICHROMATE METHOD.

The dichromate method described above was subsequently re-examined at the Waite Institute, Adelaide, and some minor modifications were introduced to give more convenient working and to overcome difficulties due to chlorides. It was found that time could be saved without loss of accuracy (*a*) by using soil ground to pass the 0·5 mm. sieve instead of the 0·13 mm. (100 mesh) sieve, and (*b*) after digesting and shaking for about 1 min. by leaving the flask to stand on an asbestos sheet without further shaking for about 30 min. With large numbers of determinations it is convenient to carry through the digestions in order and then to proceed to the titrations when the first flask is cool. The speed and reliability of the method with these modifications may be illustrated for a series of analyses on fifteen red-brown earth soils from seven typical

South Australian profiles. Thirty determinations including sampling from 3-lb. lots of 2-mm. soil and grinding were carried through comfortably in one working day. The mean difference between duplicates was about 2 per cent. of the carbon content. In Fig. 1 dichromate results are plotted against dry combustion values, kindly supplied by Mr C. S. Piper. If the results had been corrected for the mean recovery factor of about 77 per cent. the errors of analysis would have been small by comparison with the inevitable errors of field sampling in most advisory and survey work.

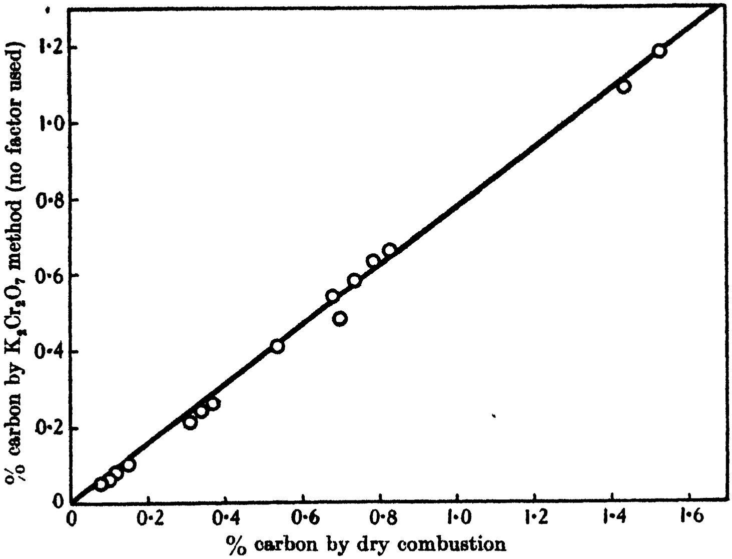

Fig. 1.

In the course of work for a co-operative investigation by a Committee of the Second Commission of the International Society of Soil Science, reported elsewhere (16), it was found from triplicate determinations on six soils that two other modifications of Schollenberger's method by Craig and Tiurin gave higher and more nearly constant recoveries between soils but less good agreement between replicates than the Walkley-Black method, with or without the above modifications. In the Craig and Tiurin modifications, as in the original Schollenberger method, the soil and chromic acid mixture is heated directly to much higher temperatures than are obtained through the heat of dilution of sulphuric acid in the Walkley-Black dichromate method. The simplicity and uniformity of treatment in the dichromate method thus compensates to some extent for the incompleteness of the oxidation, and renders the method particularly suitable for comparing series of soils of similar types.

The violence of the reaction on adding the sulphuric acid varies according to the strength of the acid. Thus, with 98 and 96 per cent. acids the maximum temperatures reached were 120 and 115° C. Although the apparent carbon content by the weaker acid was 98 per cent. of that by the stronger, care should be taken to avoid acids below 96 per cent. sulphuric acid.

Soils containing considerable amounts of chloride gave unduly high results by the dichromate method. Dark fumes were liberated and condensed on the cooler parts of the flask as a heavy red liquid, presumably chromyl chloride. Some of this vaporised and the remainder was probably hydrolysed on dilution. Direct experiments with sodium chloride showed that the dichromate was reduced quantitatively ($1C = 2O = 4Cl$). Since 12 parts of carbon are equivalent to 142 parts of chlorine, an approximate correction for the effects of chlorides may be made by assuming that the recovery of carbon is not influenced by the chloride, and subtracting 1/12th of the chlorine content of the soil from the apparent carbon content of the soil (or 1/9th of the chlorine content from the corrected carbon contents when the percentage recovery of carbon is about 75 per cent.). The results in Table IV for a Mallee soil with 0·12 per cent. chlorine show that this correction was satisfactory for the effects of added sodium chloride, until the Cl : C ratio approached 10.

Table IV. *Correction of dichromate titrations for the effects of chlorides.*

Cl %	Apparent C % by dichromate	Apparent C % minus 1/9 Cl %	C % by dry combustion
0·012	0·355	0·354	0·356
0·184	0·374	0·354	0·355
0·409	0·397	0·352	0·354
0·75	0·435	0·352	0·352
1·06	0·473	0·355	0·350
1·79	0·551	0·352	0·346
2·45	0·611	0·339	0·342
3·30	0·666	(0·299)	0·337

Where the chloride content of the soil is unknown its disturbing effect may be eliminated, at least in part, by precipitating the chloride as silver chloride. It was found that quite large amounts of silver sulphate (up to 150 gm. per litre) dissolve in concentrated sulphuric acid, though no quantitative data have been found in the literature. Sulphuric acid containing 100 gm. Ag_2SO_4 per litre gave an apparent recovery of 85 per cent. for a soil with over 4 per cent. Cl, as compared with 150 per cent. when no Ag_2SO_4 was used. The Ag_2SO_4 had only a small effect in nonsaline soils. Such large excesses of silver sulphate are probably unnecessary. If the Cl : C ratio does not exceed 1, then 25 gm. Ag_2SO_4 per litre H_2SO_4 should be sufficient to fix the chloride. For more saline soils the chloride content is a most important character of the soil and will generally be determined. The chloride may, of course, be removed by a preliminary washing with water.

It was found that nitrates up to 5 per cent. of the carbon content and carbonates up to at least 50 per cent. of the soil caused no disturbance. Manganese dioxide as an impure pyrolusite was added in amounts up to 3–4 times the carbon content without disturbance. It would appear that the high acid concentration prevents the oxidising action of the manganese dioxide.

N $FeSO_4$ in approximately *N*/2 H_2SO_4 was found to be more convenient than the more dilute ferrous ammonium sulphate previously recommended. Both solutions were kept under an atmosphere of hydrogen in stock bottles filled with self-adjusting zero burettes.

The method as at present modified is as follows:

Up to 10 gm. soil passing the 0·5 mm. sieve and preferably containing from 10 to 25 mg. carbon are weighed into a 500 ml. conical flask. 10 ml. *N* $K_2Cr_2O_7$ are run in from a burette, followed by 20 ml. concentrated H_2SO_4 (not less than 96 per cent.) from an automatic pipette. The mixture is shaken for about 1 min. and left to stand for about 30 min. About 200 ml. water are added, followed by approximately 10 ml. of 85 per cent. H_3PO_4 and 1 ml. of 0·5 per cent. diphenylamine. The colour is not always purple on adding the indicator, but nearly always turns purple or blue just before the end-point is reached. The best procedure for titration is to run in the standard *N* $FeSO_4$ solution till the colour is purple or blue, and then to continue the addition in 0·5 ml. lots until with little or no warning the colour flashes to green. 0·5 ml. of *N* $K_2Cr_2O_7$ is added and the titration finished by adding the $FeSO_4$ solution drop by drop. It has always been found possible to determine the end-point to one drop. Often the blue colour does not reform after adding the 0·5 ml. *N* $K_2Cr_2O_7$, but it soon reappears after adding a drop or two of $FeSO_4$. If more than 8 ml. of the available 10 ml. of $K_2Cr_2O_7$ are reduced the determinations should be repeated with less soil. The percentage recovery varies somewhat with the type of soil and the details of technique, and should be determined by comparisons with dry combustions on similar soils. The author found mean recoveries of about 77 per cent., or a correction factor of 1·3 (1 ml. *N* $K_2Cr_2O_7 = 1{\cdot}3 \times 3{\cdot}0 = 3{\cdot}9$ mg. C).

SUMMARY.

1. The details of the Dennstedt dry-combustion method for determining carbon in soils were described, and some simplifications suggested.

2. The Bangor modified Kjeldahl method for carbon and nitrogen in soils requires carefully standardised heating. Errors may arise from contamination of sandy soils by material abraded during grinding in iron or porcelain mills.

3. For many heavy soils the addition of water before the Kjeldahl digestion is convenient but not essential. For heavy alkaline soils with little organic matter it is advisable to grind the soil very finely and to add water.

4. The rapid dichromate titration method of Walkley and Black for soil carbon gave satisfactory approximate results. The details of the technique were improved and methods were devised for overcoming disturbances due to chlorides. The method should be useful in advisory and survey work in which the errors of soil sampling in the field are inevitably high.

The author wishes to express his thanks to Dr E. M. Crowther for his interest and assistance in this work, and to Mr C. S. Piper for supplying dry combustion data for the Australian soils.

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(*Received February 25th*, 1935.)

LEAF ANALYSIS AS A MEANS OF DIAGNOSING NUTRIENT REQUIREMENTS OF TROPICAL ORCHARD CROPS.

By F. HARDY, J. A. McDONALD and G. RODRIGUEZ.

(*Imperial College of Tropical Agriculture, Trinidad, British West Indies.*)

I. Introductory.

The possibility of employing the data obtained by chemical analysis of leaf material as a means of revealing the nutrient relationships between a crop and its environment has attracted the repeated attention of agricultural chemists ever since the days of Liebig. In general, the method has failed to provide a reliable index of nutrient relationships satisfactory in all cases, although instances may be cited where valuable evidence of specific nutrient deficiencies or excesses has been obtained by the procedure. Modern knowledge of the physiological processes involved in the uptake of nutrients from the soil by the plant, and their translocation and transformation within the plant during the various phases of growth, although still very inadequate, has indicated their great complexity, and has diminished the hope that plant analysis will supply a reliable guide to the correct nourishment of crops by controlled manuring in each and every case.

Thus, Russell has recently pointed out (1) that the "amounts of nutrient and other materials in the plant are not determined by the needs of the plant but by the quantities available. The plant roots...absorb some of everything that is soluble in the soil, not, however, in the proportion in which it exists in the soil, but in a proportion modified by certain characters of the plant...." "The final composition of the plant is therefore profoundly affected by the composition of the soil, and, except in cases of extreme deficiencies, is not a good index of the fertiliser requirements of the plant."

The relationships that exist between the plant and its environment are not simple, and factors other than nutrient supply may affect the growth and composition of the plant. Thus, a nutrient supply adequate for one level of water supply may be quite inadequate for another, and the same may hold true for other growth conditions, such as air supply.

In spite of these fundamental difficulties, the study of soil fertility, considered in its two main aspects, namely (*a*) the precise physiological needs of particular plants, and (*b*) the nutrient supplying ability of soils for the more important entities (nitrogen, phosphate, potash, lime, magnesia), has yielded valuable results in certain cases. For example, Wallace and his co-workers at Long Ashton have successfully applied plant analysis as a means of diagnosing the nutrient requirements of fruit trees in the west of England, and for distinguishing between different kinds of chlorosis due to potassium deficiency and to iron deficiency respectively. The details of their discoveries and those of other workers in this field, considered in the light of modern views regarding "nutrient balance" in the soil and the plant, have been discussed by one of us in a recent review (2).

II. SPECIFIC DIFFERENCES IN THE NUTRIENT REQUIREMENTS OF CACAO AND GRAPEFRUIT IN TRINIDAD.

An interesting case of contrasted individuality in the nutrient requirements of two important tropical fruit crops, namely cacao and grapefruit, was revealed during the progress of our ecological investigations in Trinidad. Studies of the cacao crop were initiated in 1931 as part of the College Scheme of Cacao Research, and are fully described in four *Annual Reports*, published in 1932, 1933, 1934 and 1935. Studies of the grapefruit crop along independent lines were begun in early 1934, and have been described in seven typescript progress reports; the main findings are summarised in an article published in *Tropical Agriculture* in July 1935 (3).

(1) *Soil characters.*

The cacao and grapefruit soils of Trinidad are varied in origin and composition. Most of them are alluvial soils, occupying the shallow basins of hill streams, or forming narrow levees bordering the banks of small rivers that traverse the undulating plains. The particular soils considered in this article are four in number. Two of them (A and B) occur at River Estate, situated in the Diego Martin Valley in the western part of the Northern Range; they are typical river alluvial soils. The third (D), which is also alluvial, occurs at the St Augustine Experiment Station, situated at the mouth of a more easterly valley of the Northern Range, whilst the fourth (C) occurs near Rio Claro in the southern part of Trinidad, and is derived from sedimentary Princes Town Marl, of Miocene age. The main features of these four soils are indicated by the laboratory data collected in Table I.

Table I. *Comparison of cacao and grapefruit soils, Trinidad.*

(Laboratory data for 1 ft. profiles.)

Depth of sampling inches	Sticky point (P) %	Sand content (S) %	Index of texture (I.T.) $P-S/5$	Reaction pH	Org.* matter (O.M.) %	Total nitrogen (N) %	C/N ratio	Electrical conductivity (mhos × 10^{-6})		Available phosphate (P_2O_5) Truog p.p.m.	Available (exchange) potash (K_2O) p.p.m.	Exchange lime (CaO) %	Saturation capacity (CaO) %	Degree of saturation %
								Available nutrients	Rate of solution					
(A) Cacao; River Estate; manurial experiment; control plots.														
3	35·9	55·8	25	5·7	2·70	0·16	9·6	55	35	11	65 64	0·142	0·428	33·3
6	34·8	53·1	25	5·5	1·87	0·11	10·3	40	23	12				
12	34·1	47·5	25	5·3	1·28	0·08	9·6	35	15	8	51	—	—	—
(B) Grapefruit; River Estate; neighbouring field to last.														
3	31·4	71·5	17	5·2	2·56	0·13	11·2	50	12	28	51 —	0·042	0·352	12·1
6	30·2	72·2	16	5·1	1·88	0·12	9·5	44	12	14				
12	28·9	67·5	16	5·0	0·99	0·07	8·4	30	6	10	—	—	—	—
(C) Cacao; Rio Claro.														
3	56·6	16·1	53	6·5	5·75	0·33	9·9	136	209 51	11	206	0·640	0·904	70·9
6	49·2	9·6	47	6·2	1·96	0·15	7·3	64						
12	48·0	5·1	47	5·6	0·59	0·08	4·1	36	10	—	—	—	—	—
(D) Grapefruit; St Augustine; manurial experiment; control plots.														
3	33·5	44·3	25	6·4	2·24	0·15	8·7	58	27	34	132 39	0·171	0·309	55·8
6	33·2	42·5	25	6·3	1·71	0·13	7·7	41	32	20				
12	32·4	40·2	24	6·3	1·47	0·11	7·3	36	33	22	—	—	—	—

* Corrected.

The three alluvial soils (A, B, D) are sandy loams, naturally highly acidic in reaction. The St Augustine soil (D) was limed some years ago whilst the area was growing a previous crop of sugar-cane, and is now only slightly acidic. The Rio Claro sedimentary soil (C) is a slightly acidic clay. The content of nitrogenous organic matter is medium high, but diminishes rapidly below the surface 3 in. layer. Its carbon-nitrogen ratio is variable, being highest in the River Estate grapefruit soil (B), which was previously growing cacao, whose surface litter has not yet completely decomposed. The nutrient status, as measured by the electrical conductivity of water extracts (Atkins' method), is medium low, except in the Rio Claro soil, in which it is high in the surface layer rich in nitrogen. Available phosphate contents are low and probably very inadequate; available potash contents are medium in amount and also probably inadequate, except in the Rio Claro soil, which is fairly well supplied with potash. The lime status of the two River Estate soils is poor, but that of the limed St Augustine soil and of the Rio Claro marl soil is fairly good.

(2) *Composition of leaf ash.*

The four soils are thus fairly similar in their physical attributes and their nutrient status, except that one cacao soil and one grapefruit soil possess a higher lime status than the other two corresponding soils. Nevertheless, the composition of the leaf ash of cacao is entirely different from that of grapefruit supported by these soils (Table II). Comparing first the River Estate cacao and grapefruit leaf material, produced by trees growing in neighbouring fields of acidic soil, it will be noted that, although the lime status of the cacao soil is somewhat higher than that of the grapefruit soil, yet *the lime content of the leaf ash of cacao is very much less than that of grapefruit*, whilst the potash content is correspondingly greater. Comparing next the Rio Claro cacao leaf material with that of grapefruit at St Augustine, it will similarly be noted that the lime content of the cacao leaf ash here also is very much lower than that of grapefruit, and potash content very much higher. Similar difference will also be noted in the nitrogen contents.

Evidently the grapefruit tree is markedly calcicolous, whilst the cacao tree appears, on the contrary, not to be calcicolous. When grown in soils of comparable lime status, the leaf ash of grapefruit contains more than twice as much lime as that of cacao. Even when grown in soil very deficient in available lime, the grapefruit tree seems to be able to concentrate a considerable amount of lime in its leaves. Leaf lime content

Table II. *Comparison of chemical composition of cacao and grapefruit leaves, Trinidad.*

(Analytical data for ash components.)

	% total ash										
		Composition of leaf ash				Nutrient ratios					
Ash % dry matter	Nitrogen N	Phosphate P_2O_5	Potash K_2O	Lime CaO	Magnesia MgO	N/P_2O_5	N/K_2O	K_2O/P_2O_5	CaO/K_2O	CaO/MgO	$K_2O+CaO+MgO$
(A) Cacao; River Estate; manurial experiment; control plots.											
6·72	33·04	9·46	32·18	12·06	11 06	3·49	1·03	3·40	0·37	1·09	55·30
(B) Grapefruit; River Estate; neighbouring field to last; unmanured.											
8·51	27·61	4·96	28·46	24·34	12·93	5·57	0·97	5·73	0·86	1·88	65·73
(C) Cacao; Rio Claro.											
9·81	22·82	3·59	20·74	20·94	10·90	6·47	1·12	5·82	1·06	1·92	52·58
(D) Grapefruit; St Augustine; manurial experiment; control plots.											
12·46	17·50	6·63	7·39	42·70	6·62	2·64	2·38	1·11	5·78	6·45	56·71
For comparison.											
Cacao; "Ideal"; standard tree; Trinidad.											
9·90	23·74	5·05	20·16	—	—	4·70	0·89	5·28	—	—	—
Grapefruit; "Ideal"; average trees; California.											
17·7	13·5	2·1	7·2	48·4	4·5	6·4	1·9	3·4	6·7	10·8	60·1

bears a strict and highly significant reciprocal relationship with leaf potash content.

(3) *Effects of simple manuring.*

The addition of single manurial substances has affected differently the composition of the leaves of cacao and of grapefruit growing in treated plots at River Estate and at St Augustine, where large-scale manurial experiments are being conducted on the two crops. The main differences are shown by the analytical data for leaf-ash composition presented in Table III. The annual treatments consist of applications of ammonium sulphate, superphosphate and potassium sulphate in the following quantities:

	Quantities per tree	
	River Estate cacao lb.	St Augustine grapefruit lb.
(Nitrogen) sulphate of ammonia	4	1½
(Phosphate) superphosphate of lime	2	1½
(Potash) sulphate of potash	2	6

The values for leaf-ash components set out in the table are means of the results of determinations made on leaf samples collected by a tested procedure proved to provide representative material.

In each case, the manuring has considerably increased the content of leaf ash, but it has affected the actual composition of the leaf ash of grapefruit very much more than that of cacao. The percentage increases and decreases in the amounts of the different leaf components, caused by the manuring, are given in the bottom part of Table III. The figures clearly indicate the very much greater changes in leaf composition of grapefruit compared with cacao, as a result of manuring.

Effects on individual leaf components.

(*a*) *Nitrogen manure.* The addition of nitrogen alone has somewhat diminished the relative leaf nitrogen content of cacao, but has very greatly increased it in the case of grapefruit. It has depressed the leaf phosphate contents in both cases, slightly in the case of cacao, but very greatly in the case of grapefruit, even though the dressing of superphosphate applied to grapefruit was relatively very large. It has slightly increased leaf potash content of cacao, but greatly diminished it in the case of grapefruit. The effect on leaf potash is reciprocated in the leaf lime contents.

Table III. *Effects of single manurial treatments on chemical composition of cacao and grapefruit leaves.*

	Ash % dry matter	Composition of leaf ash					Nutrient ratios					$K_2O+CaO+MgO$
		N	P_2O_5	K_2O	CaO	MgO	N/P_2O_5	N/K_2O	K_2O/P_2O_5	CaO/K_2O	CaO/MgO	
(A) Cacao; River Estate.												
No manure	6·72	33·04	9·46	32·18	12·05	11·07	3·49	1·03	3·40	0·37	1·09	55·30
Nitrogen	7·13	31·48	9·21	33·67	11·93	11·09	3·42	0·93	3·66	0·35	1·08	56·69
Phosphate	7·03	32·16	9·78	32·76	12·04	11·16	3·29	0·98	3·35	0·37	1·08	55·96
Potash	7·50	29·79	8·18	33·75	11·70	10·79	3·64	0·88	4·13	0·35	1·08	56·24
"Ideal"	9·9	23·7	5·0	26·7	—	—	4·7	0·9	5·3	—	—	—
(B) Grapefruit; St Augustine Station.												
No manure	12·46	17·50	6·63	7·39	42·70	6·62	2·64	2·38	1·11	5·79	6·45	56·71
Nitrogen	13·00	22·78	3·08	3·55	44·10	6·71	7·40	6·42	1·15	12·43	6·57	54·36
Phosphate	13·52	17·97	6·23	5·16	44·50	5·62	2·89	3·48	0·83	8·63	7·92	55·28
Potash	13·47	16·04	6·04	16·02	37·48	4·96	2·65	1·00	2·65	2·34	7·66	58·46
"Ideal"	17·7	13·5	2·1	7·2	48·4	4·5	6·4	1·9	3·4	6·7	10·8	60·1

Quantitative effects of simple manuring.

		% increase or decrease					Numerical change in ratio values					
(A) Cacao.												
Nitrogen	+ 6	− 5	− 3	+ 5	− 1	− 2	−0·1	−0·1	+0·3	0·0	0·0	—
Phosphate	+ 4	− 2	+ 4	+ 2	− 1	− 1	−0·2	0·0	−0·1	0·0	0·0	—
Potash	+12	−10	−12	+ 5	− 2	− 4	+0·2	−0·1	+0·7	0·0	0·0	—
(B) Grapefruit.												
Nitrogen	+ 4	+30	−53	− 52	+ 3	− 1	+4·8	+4·0	0·0	+6·6	+0·1	—
Phosphate	+ 8	+ 3	− 6	− 30	+ 4	−15	+0·3	+1·1	−0·3	+2·8	+1·5	—
Potash	+ 8	− 8	− 9	+117	−12	−24	0·0	−1·4	+1·5	−3·5	+1·2	—

(*b*) *Phosphate manure.* The addition of phosphate alone has affected oppositely but slightly leaf nitrogen contents and leaf phosphate contents. It has actually reduced leaf phosphate in grapefruit. It has increased leaf potash content in cacao, but very greatly diminished it in grapefruit, just as did nitrogenous manure. Lime contents show reciprocal relations to potash contents, but magnesia contents show direct relations to potash contents.

(*c*) *Potash manure.* The addition of potash alone has markedly diminished leaf nitrogen and leaf phosphate contents in each case, especially in cacao, but has increased leaf potash contents, extremely greatly in the case of grapefruit (over 100 per cent.), but only slightly in the case of cacao, again with reciprocal effects on leaf lime contents, but with opposite effects on leaf magnesia.

The most striking differences between cacao and grapefruit indicated by the effects of single manuring on leaf ash composition, under the conditions of these experiments, are (*a*) the much more pronounced effect of nitrogenous manuring on nitrogen, phosphate and potash contents of grapefruit leaf, (*b*) the more pronounced effect of potash manuring on nitrogen and phosphate contents of cacao leaf, and (*c*) the more pronounced effect of potash manuring on the potash content of grapefruit leaf.

Comparison with selected standards.

In order to determine which of the different single manurial treatments have *improved* the nutrient status of the soil, data for leaf-ash composition and nutrient ratios obtained by chemical analysis of leaf material produced by selected standard "ideal" cacao and grapefruit trees are included in Table III. The "ideal" cacao tree was selected in Trinidad from a field of good reputation, where high yields have been recorded for many years. Unfortunately, no similar "ideal" grapefruit orchard could be chosen in Trinidad, because none of the grapefruit areas is sufficiently old, nor presents a long enough record of high-bearing capacity, to serve as reliable standard. Consequently, recourse was made to published analyses in the literature of *Citrus*, and an average was taken for satisfactory, high-yielding, healthy Californian trees. The employment of such a standard for grapefruit is open to serious objection, but, under the circumstances, and provided analogy is not over-stressed, the selected "ideal" may serve present purposes.

When the chemical composition and the nutrient ratios of leaf ash corresponding to the three single manurial treatments are compared with those of the "ideals", it appears that the single *potash* treatment has conferred most benefit, both for cacao and for grapefruit, although none of the single treatments is completely satisfactory.

(4) *Effects of compound manuring.*

The addition of combinations of manures, both paired and tripled, has also differently affected the composition of cacao and grapefruit leaves, as indicated by the analytical data in Table IV. The manuring has greatly increased the total ash content of cacao leaves, but has increased it in the case of grapefruit in two instances only.

Effects on individual leaf components.

(*a*) *Nitrogen* plus *phosphate manure.* The addition of nitrogen *plus* phosphate has increased nitrogen content of leaf in both cases, but much the greater in the case of grapefruit (nearly 70 per cent. increase). It has slightly increased leaf phosphate content of cacao, but greatly diminished it in the case of grapefruit, just as did nitrogen alone. It has decreased leaf potash contents, slightly for cacao, and very greatly for grapefruit, with reciprocal effect on leaf lime contents.

(*b*) *Nitrogen* plus *potash manure.* The addition of nitrogen *plus* potash has decreased leaf nitrogen and phosphate contents in both cases, and especially phosphate in the case of grapefruit. It has appreciably increased potash content in both cases.

(*c*) *Phosphate* plus *potash manure.* The addition of phosphate *plus* potash has markedly decreased nitrogen content of leaf in both cases, but has decreased phosphate content only in the case of cacao. It has greatly increased leaf potash contents, especially in the case of grapefruit (67 per cent.), and has correspondingly diminished lime content and magnesia content.

(*d*) *Nitrogen* plus *phosphate* plus *potash manure.* The addition of nitrogen *plus* phosphate *plus* potash has decreased the leaf nitrogen content of cacao, but has greatly increased that of grapefruit. It has decreased leaf phosphate content in each case, especially that of grapefruit. It has only slightly affected leaf potash content, and accordingly only slightly leaf lime (and leaf magnesia) contents.

Comparing the effects of the three paired treatments and the triple treatment on nutrient ratios, it will be noted that the most effective treatments have apparently been the phosphate *plus* potash treatment in the case of cacao, and the nitrogen *plus* potash treatment in the case of grapefruit, in that these treatments respectively have brought the ratio values nearest to the "ideal" standards.

Table IV. *Effects of double and triple manurial treatments on chemical composition of cacao and grapefruit leaves.*

	Ash % dry matter	N	Composition of leaf ash				Nutrient ratios					K_2O + CaO + MgO
			P_2O_5	K_2O	CaO	MgO	N/P_2O_5	N/K_2O	K_2O/P_2O_5	CaO/K_2O	CaO/MgO	
(A) Cacao; River Estate.												
No manure	6·72	33·04	9·46	32·18	12·05	11·07	3·49	1·03	3·40	0·37	1·09	55·30
Nitrogen + phosphate	6·72	34·34	10·11	29·88	13·90	11·43	3·40	1·15	2·96	0·47	1·22	55·21
Nitrogen + potash	7·58	30·17	8·98	37·05	10·99	10·13	3·35	0·81	4·13	0·30	1·08	58·17
Phosphate + potash	7·62	29·46	8·77	36·87	9·94	10·20	3·36	0·80	4·30	0·27	0·97	57·01
Nitrogen + phosphate + potash	7·46	30·79	8·65	33·93	12·04	10·74	3·56	0·91	3·92	0·35	1·12	56·71
"Ideal"	9·9	23·7	5·0	26·7	—	—	4·7	0·9	5·3	—	—	—
(B) Grapefruit; St Augustine Station.												
No manure	12·46	17·50	6·63	7·39	42·70	6·62	2·64	2·38	1·11	5·79	6·45	56·71
Nitrogen + phosphate	11·86	29·53	4·38	2·95	47·16	6·57	6·74	10·00	0·67	15·95	7·17	56·68
Nitrogen + potash	14·30	17·13	3·02	7·87	45·06	5·03	5·68	2·18	2·61	5·73	8·96	57·96
Phosphate + potash	15·80	15·00	6·72	12·38	33·62	5·33	2·23	1·21	1·85	2·72	6·31	51·33
Nitrogen + phosphate + potash	12·20	21·80	4·15	7·42	43·92	6·43	5·26	2·94	1·79	5·86	6·84	57·77
"Ideal"	17·7	13·5	2·1	7·2	48·4	4·5	6·4	1·9	3·4	6·7	10·8	60·1

Quantitative effects.

	% increase or decrease							Numerical change in ratio values				
(A) Cacao.												
Nitrogen + phosphate	0	+ 4	+ 7	- 7	+15	+ 3	-0·1	+0·1	-0·4	+0·1	+0·1	—
Nitrogen + potash	+13	- 9	- 5	+15	- 9	- 8	-0·1	-0·2	+0·7	-0·1	0·0	—
Phosphate + potash	+13	-11	- 7	+15	-18	- 8	-0·1	-0·2	+0·9	-0·1	-0·1	—
Nitrogen + phosphate + potash	+11	- 7	- 9	+ 5	0	- 3	+0·1	-0·1	+0·5	0·0	0·0	—
(B) Grapefruit.												
Nitrogen + phosphate	- 5	+69	-34	-60	+10	- 1	+4·1	+7·6	-0·4	+10·1	+0·7	—
Nitrogen + potash	+15	- 2	-54	+ 6	+ 5	-24	+3·0	-0·2	+1·5	-0·1	+2·5	—
Phosphate + potash	+27	-14	+ 1	+68	-21	-19	-0·4	-1·2	+0·7	-3·1	-0·1	—
Nitrogen + phosphate + potash	- 2	+25	-37	0	+ 3	- 3	+2·6	+0·6	+0·7	+0·1	+0·4	—

(5) *Application of Thomas' rule.*

As a result of detailed investigations of the effects of manurial treatments on the chemical composition of fruit trees, Thomas (4) has propounded a generalisation which may possibly be applicable to the cacao and grapefruit results recorded in this article, namely that the addition of any two of the components, nitrogen, phosphate and potash, *to a soil initially deficient in them*, results in a decrease in the uptake of the third. This may upset the "nutrient balance", and may consequently cause diminished growth and yield.

Assuming that decreased uptake of soil nutrients is reflected in the chemical composition of the leaves (an assumption which should not necessarily be accepted without definite proof), the following conclusions are reached regarding the initial nutrient status of the two soils supporting cacao and grapefruit:

(A) *Cacao soil.* Each of the paired treatments has somewhat diminished the content of the remaining component in the leaf, so that the soil shows general nutrient deficiency, though the data do not warrant the stressing of any one component more than another.

(B) *Grapefruit soil.* Each of the paired treatments has very greatly diminished the leaf content of the third component, especially potash and nitrogen. Hence this soil appears to be particularly deficient in these two nutrients, and the tree should benefit markedly by their addition to the soil.

(6) *General conclusions.*

The chemical composition and nutrient ratios of the leaf material of cacao and of grapefruit reveal great differences in their soil nutrient relationships and their response to manurial treatment. By merely comparing the analytical data with those for standard leaf material produced by "ideal" trees, the particular manurial treatments which should confer most benefit may readily be identified. So far the methods employed for this purpose have been qualitative, and have solely involved chemical analysis of representative leaf material.

III. QUANTITATIVE RELATIONSHIPS BETWEEN LEAF COMPOSITION AND RESPONSE TO MANURING OF CACAO AND GRAPEFRUIT.

The possibility of further testing the general qualitative conclusions based on considerations of the results of leaf analysis, as set out in the previous section, is presented and rendered feasible by the fact that quantitative yield data and growth measurements have been systematically recorded for the two crops during the conduct of the field manurial experiments. The experiments may best be considered in turn.

(a) *Cacao manurial experiment, River Estate.*

The experiment (5) consists of a 4 × 4 Latin square, having sixteen main plots, each containing sixteen cacao trees; guard rows separate the plots. The plot treatments are (1) no manure, (2) nitrogen, (3) potash, and (4) nitrogen *plus* potash, with superimposed half-plot treatments of phosphate. The trees were 30 years old at the beginning of the experiment, and manures have been applied twice in successive years. The yield data have been presented and fully considered by Pound and de Verteuil (6). The following yields, due to the different treatments, were recorded for the 1935 crop:

Treatment	Weight of wet cacao bean (kg.)	Number of cacao pods per tree
Control	72	29
Nitrogen	73	26
Phosphate	78	26
Potash	80	29
Nitrogen + phosphate	56	21
Nitrogen + potash	95	32
Phosphate + potash	110	42
Nitrogen + phosphate + potash	86	35

Greatest benefit was given by potash manure, especially in the presence of phosphate. To quote Pound and de Verteuil, "without doubt, on River Estate, potash could be applied up to the point when phosphate would be the limiting factor, and then both may be applied theoretically until it may even be beneficial to add nitrogen as well".

Relationship between yield and leaf composition.

In order to relate leaf composition to crop yield, the data obtained by chemical analysis of representative leaf material (see Table III) were compared statistically with the yield data compiled by Pound and de Verteuil. Coefficients of correlation are given in Table V, taken in part from a full account of the chemical aspects of this experiment previously compiled by two of us (7).

The statistical evidence in Table V shows that a remarkably close relationship holds between leaf composition and quantitative yield of cacao in this experiment. Highly significant positive correlations with yield obtain particularly for (*a*) total ash in leaf, (*b*) potash content of leaf, (*c*) potash/phosphate ratio, and (*d*) nitrogen/phosphate ratio, whilst significant negative correlation with yield obtains particularly for nitrogen/potash ratio. Increased yields are evidently associated with increased potash content of leaf and decreased nitrogen and phosphate contents, but the best indices of yield appear to be the leaf nutrient ratios, particularly a low nitrogen/potash ratio, a high potash/phosphate ratio, and a high nitrogen/phosphate ratio.

Table V. *Correlation of leaf components and yield of cacao, River Estate manurial experiment.*

Index of leaf composition	Correlation coefficients (r) Yield expressed as (A) Weight of wet cacao	(B) Number of cacao pods
Leaf ash basis:		
Total ash content and yield	*+0·583*	*+0·522*
Nitrogen content and yield	*−0·646*	*−0·672*
Phosphate content and yield	*−0·483*	*−0·575*
Potash content and yield	*+0·425*	+0·363
Lime content and yield	*−0·476*	—
Nutrient ratios:		
Nitrogen/phosphate ratio and yield	*+0·469*	*+0·538*
Nitrogen/potash ratio and yield	*−0·634*	*−0·573*
Potash/phosphate ratio and yield	*+0·533*	*+0·513*
Correlations between individual leaf-ash components:		
Nitrogen and phosphate	*+0·819*	
Nitrogen and potash	*−0·479*	
Phosphate and potash	−0·218	
Lime and nitrogen	+0·324	
Lime and phosphate	+0·100	
Lime and potash	*−0·794*	

NOTE. Values for correlation coefficient (r) exceeding 0·409 indicate significant relationships, with a minimum probability of 50 to 1. Significant correlations are italicised in the table.

The data at the bottom of Table V show the high reciprocal relationship between leaf lime and leaf potash contents which has already been stressed. They also show a lesser degree of reciprocal relationship between nitrogen and potash, and a high positive correlation between nitrogen and phosphate in the leaf material of cacao produced by the manured plots.

(2) *Effects of manurial treatments on chemical composition of leaf material of cacao.*

The values for individual chemical components (nitrogen, phosphate and potash) determined in the leaf (Tables III and IV), and the values for different nutrient ratios have been statistically examined by Fisher's Analysis of Variance method. The differential results are summarised in

Table VI, and indicate which differences in chemical components are significant. Statistical analysis was possible in this case since leaf material from *each* of the replicated plots was chemically analysed (7).

Table VI. *Effects of manurial treatments on chemical composition of cacao leaves, River Estate. (Differences in mean composition of leaves of trees in differently treated plots.)*

Manurial treatments	% ash in dry matter	N %	Composition of ash P_2O_5 %	K_2O %	Nutrient ratios N/P_2O_5	N/K_2O	K_2O/P_2O_5
Control	0	0	0	0	0	0	0
Nitrogen	+0·37	−1·5	−0·28	+1·6	−0·10	−0·11	−0·27
Phosphate	+0·32	−0·7	+0·37	+0·6	−0·20	−0·05	−0·08
Potash	+*0·75*	−*3·5*	−*1·25*	+1·6	+0·16	−*0·17*	+*0·67*
Nitrogen + phosphate	−0·03	+1·5	+0·70	−2·2	−0·12	+0·11	−0·46
Nitrogen + potash	+*0·85*	−*2·7*	−0·50	+*4·8*	−0·12	−*0·24*	+*0·69*
Phosphate + potash	+*0·90*	−*3·7*	−0·73	+*4·6*	−0·13	−*0·25*	+*0·85*
Nitrogen + phosphate + potash	+*0·72*	−2·2	−0·80	+1·8	+0·08	−*0·14*	+*0·50*
S.E. of difference between two means	0·26	1·16	0·46	1·0	0·15	0·06	0·18
S.E. × t = difference required for significance	0·57	2·53	1·00	2·18	0·33	0·12	0·39

Values represent mean differences on leaf composition due to each manurial treatment. Differences must be greater than 2·18 times the S.E. in order to be significant with a minimum probability of 20 : 1.

Differences which are significant are indicated by italic type.

The statistical results reveal the following significant effects of manurial treatments on leaf composition of cacao (7):

(1) *Potash manuring* alone has (*a*) increased the total ash, (*b*) decreased the nitrogen and phosphate contents, (*c*) decreased the nitrogen/potash ratio, and (*d*) increased the potash/phosphate ratio. These effects have produced nutrient ratio values more closely approaching those of the standard, high-yielding "ideal" cacao, thus demonstrating that potash manuring causes a more evenly balanced nutrient supply to the cacao tree at the River Estate site.

(2) Nitrogen manuring alone and phosphate manuring alone have produced no significant effects on leaf composition.

(3) Nitrogen *plus* potash manuring and phosphate *plus* potash manuring have produced significantly beneficial effects through increased contents of potash of leaf.

(4) Nitrogen *plus* phosphate manuring has removed the nutrient ratios in the leaf further from the standards, and has apparently produced a very unbalanced nutrient supply.

The evidence thus derived from a statistical analysis of the chemical leaf data proves that potash is markedly deficient in the soil of the site of the experiment, and that available potash is the limiting factor for yield. Available nitrogen and phosphate appear to be present in the soil in adequate quantities relative to potash. Although beneficial effects have resulted mainly from the application of potash manure, evidence has also been obtained by this analysis that additional benefit might be derived from small dressings of nitrogenous and phosphatic manures, applied together with potash. The amounts of nitrogen and of phosphate added by the dressings employed in this experiment seem to be excessive, relative to potash.

These findings, based on chemical leaf analysis, agree closely with those adduced by Pound and de Verteuil from a study of the yield data, thus substantiating the claim that chemical analysis of leaf material may furnish reliable evidence of nutrient requirements of crop and soil, provided a suitable standard of comparison, namely leaf material from a healthy, high-yielding tree, is available.

(*b*) *Grapefruit manurial experiment, St Augustine Station.*

The experiment (8) consists of a 4 × 4 Latin square, with four subplots in each square, arranged at random. Each subplot contains nine grapefruit trees, arranged in a 3 × 3 Latin square. There are three types of tree, consisting of Marsh Seedless scions budded onto Sour Orange, Wild Grapefruit and Rough Lemon stock respectively. Guard rows surround each subplot. The treatments are (A) a basal treatment on each plot consisting of four subplots, namely (1) no manure, (2) phosphate, (3) potash, (4) phosphate *plus* potash; (B) superimposed treatments, namely (5) no manure, (6) unit nitrogen, (7) double nitrogen, (8) unit nitrogen *plus* pen manure. The trees were planted out from the nursery when about 1½ years old, and manures were applied from the start, namely, in December 1932, so that they have been added thrice in successive years.

(1) *Relationship between growth rate and leaf composition.*

The trees have not yet come into commercial bearing, so that, unfortunately, no yield data are available. Nevertheless, measurements of growth increments have been made twice a year for 2½ years. They consist of girth measurements at heights of 16 and 36 cm. from ground level, and measurements of average height and of average spread. The growth data have been fully considered by Williams and Gregory (9). Growth increments (expressed as mean percentages of the dimensions of the trees of the control plots), due to the different manurial treatment for grapefruit budded on to Sour Orange stock, are:

Treatment	% growth increment over control	Order of effectiveness
Control	0	—
Nitrogen	25	5
Phosphate	24	6
Potash	68	4
Nitrogen + phosphate	11	7
Nitrogen + potash	112	1
Phosphate + potash	87	3
Nitrogen + phosphate + potash	96	2

In this experiment, nitrogen *plus* potash manure has produced greatest growth. Other potash treatments, including potash alone, have also given considerable increases, whilst nitrogen and phosphate manures, both singly and in combination, have given small responses, equal only to about one-fifth to one-third those due to manures containing potash. (Pen manure treatments have not been considered in this summary.)

(2) *Effects of manurial treatments on chemical composition of leaf material of grapefruit (Marsh Seedless budded onto Sour Orange stock).*

The values for individual chemical components (nitrogen, phosphate and potash) determined in the leaf, and for different nutrient ratios have already been presented in Tables III and IV. (Only leaf material from trees budded on to Sour Orange stock was chemically analysed; analyses of material from trees treated with pen manure are not included.)

Unfortunately, samples of leaf material were not collected from the individual replicated plots, but only composite samples of the different group treatments were taken, so that the Analysis of Variance method cannot be applied to the grapefruit data. Nevertheless, the order of effectiveness on growth rate of the different manurial treatments is nearly identical with the order of arrangement derived by consideration of chemical composition and nutrient ratios of the leaf material in comparison with the Californian standard. In each case, the nitrogen *plus* potash treatment was placed first in the list, followed closely by the nitrogen *plus* phosphate *plus* potash treatment, whilst the treatments lacking potash come near the bottom of the list. There is therefore some justification for employing the Californian standard for the purpose of gauging the effectiveness of the different manurial treatments on the growth of grapefruit in this experiment.

General conclusions.

Comparison by statistical analysis or by simple inspection of yield and growth data with data for chemical composition and nutrient ratios of leaf material of cacao and grapefruit trees treated with different manures, yields reliable evidence of the relative effectiveness of the manurial treatments. Consequently, chemical analysis of suitable leaf material collected from these tree crops should enable an assessment of the nutrient status of the soil to be made, and should render feasible the diagnosis of nutrient deficiencies or excesses, *provided reliable standards of comparison are available.* Such standards may be selected either from "ideal" trees of known high-yielding capacity and good reputation, or alternatively from trees giving the highest yields and growth rates in manured plots comprising a suitably designed field experiment. Either standard of comparison might serve the purposes of a general ecological investigation, but greatest reliance should preferably be placed on the results of a statistical analysis of yield and chemical analytical data derived from a properly planned field manurial experiment.

IV. SUMMARY.

1. Attention is drawn to the remarkable dissimilarity between the cacao and the grapefruit tree in their nutrient requirements, indicating that grapefruit is calcicolous in physiological habit. This conclusion is mainly based on a consideration of the results of chemical analysis of representative leaf material produced by trees grown on soil of known chemical and physical characteristics, under the same climatic conditions in Trinidad.

2. The nutrient relationships that obtain between the cacao and the grapefruit tree respectively and the soil in which they are growing have been gauged by means of chemical analysis of representative leaf material.

3. For this purpose; leaf material was obtained from trees growing on the differently manured plots of two large-scale field experiments in Trinidad.

4. Three different procedures have been employed in the utilisation of the leaf analytical data for the purpose of assessing the nutrient requirements of the trees and the nutrient status of the soil.

5. The three procedures are:

(A) The employment of standards of comparison consisting of "ideal" trees. In the case of cacao, a healthy tree of known high-yielding capacity was used. In the case of grapefruit, no such standard was available, so that recourse was made to published analyses of Californian *Citrus* trees presumed to be satisfactory as regards growth and yield.

(B) Statistical comparison between yield data and data for chemical composition of leaf material; this procedure was applied to the case of cacao.

(C) Comparison between growth increment data and data for chemical composition of leaf material; this procedure was applied to the case of grapefruit, since no yield data were available in this instance.

6. In making the comparisons, the nutrient ratios of the different components of the leaf ash (phosphate, potash, lime, magnesia) and of leaf nitrogen were found to be particularly useful.

7. Knowledge gained by the procedures described should prove serviceable in diagnosing nutrient deficiencies or excesses in cacao and grapefruit soils occurring under similar climatic conditions as those obtaining in Trinidad. In this manner, chemical analysis of leaf material may provide a reliable ecological instrument for the study of crop production.

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(*Received June 24th*, 1935.)

SEED DISINFECTION.

I. AN OUTLINE OF AN INVESTIGATION ON DISINFECTANT DUSTS CONTAINING MERCURY.

By W. A. R. DILLON WESTON, Ph.D.

(*School of Agriculture, Cambridge.*)

and J. R. BOOER, B.Sc.

(With Plate VIII and Four Text-figures.)

Introduction.

It is probable that the smut diseases of cereals have been known since they became cultivated crops, and that throughout this period attempts have been made to minimise the losses that they have caused. We need not elaborate these control measures here since we are not probing the historical aspect—every era has had its fashion and this has been dictated by superstition, credulity, speculation or scientific evidence.

The disinfection of grain to prevent such diseases as bunt of wheat, covered smut of barley and smut of oats has been practised in the past by many progressive farmers and is now a well-established principle of plant hygiene. In recent years many farmers have changed from the wet treatments to dry, the latter consisting of disinfectant dusts that contain salts of mercury as their fungicidal basis. These dusts are now widely used, and by many are considered to be an advance on the older wet treatments, since, when correctly applied, they give control of leaf spot of oats and leaf stripe of barley—two diseases that were not adequately controlled by a wet treatment such as a solution of the gas formaldehyde in water.

Work on seed disinfection has been carried out at the School of Agriculture, Cambridge, since 1923, and the various methods and materials used for this purpose have been investigated; recently, during the past four years, the writers have co-operated in a detailed investigation of the fungicidal nature of mercuric salts and their use, when compounded as dusts, as seed disinfectants. It is this investigation that is now considered.

A survey of the literature on mercurial dressings shows the absence of any systematic investigation. The large bulk of literature when consulted can be divided into three classes; first the study of an individual mercury compound tested for the control of one disease, second the testing of proprietary products of undisclosed composition, and third a somewhat extensive literature on patents.

We do not propose to deal in detail with the work of earlier investigators, but reference should be made to Martin, *Scientific Principles of Plant Protection* (1928), where on p. 198 the following observations are made:

"Strictly speaking, the true organic mercurials are those in which the mercury atom is attached direct, by one or both valency bonds, to carbon atoms. It has been shown that, in general, those organic mercurials which possess high bactericidal powers are those of the type: R-Hg-X where R represents a benzene ring with or without substituent groups and X represents a second group which, in those compounds which have so far been employed as seed disinfectants, appears to be either a hydroxyl group, a cyanide, or possibly a halogen atom."

On p. 200:

"Young, White and Swartz found the sodium salt of hydroxy-mercuri-dibrom fluorescein, with a content of only 26 per cent. mercury, is three times as toxic as the sodium salt of hydroxy-mercuri-fluorescein which contains 33 per cent. of mercury. It is probable that the fungicidal mercurials will show a similar dependence on the nature of the groups to which the mercury is attached. Under such circumstances the determination of the mercury content of such products will not give a true idea of the value of the material as a seed disinfectant."

This is probably the first critical suggestion of the possible existence of a relationship between fungicidal properties and chemical composition.

OBJECTS OF THE INVESTIGATION.

The present work, which has entailed approximately 10,000 field plots, was started in 1932 with the following objects:

(1) To produce an effective seed disinfectant of known composition.

(2) If possible, to establish a relationship between chemical composition and fungicidal power.

(3) To find out how seed disinfectants act.

At this stage, it can be said that the investigation has met with some success on the first two points, but that on the third it is not possible, as yet, to indicate evidence of material progress.

PRELIMINARY TRIALS.

In the early experiments, dusts were prepared containing most of the known inorganic compounds of mercury and a selection from the organic compounds and these were compounded with the same filler. Five and ten per cent. of the appropriate salts were used in these dusts and they were employed to control leaf spot of oats. The dosage was at the rate of 2 oz. to the bushel of grain and the dusting operation was carried out for a period of 1 min. Each plot consisted of a rod row and each of these was replicated five times. Certain proprietary dusts were included for the purpose of comparison. As the resulting estimates were made by recording the disease present in 200 oat seedlings per plot, there was therefore a total count of 1000 plants per treatment. In these preliminary experiments the plots were not randomised, but each treatment was spaced at regular intervals throughout the field and every tenth plot was an untreated one. Although this procedure has varied slightly a somewhat similar one has been adopted when investigating the control of other diseases and also in the various problems that have arisen during the course of the work. In several of the later trials eight replicates have been made and these have been randomised. In the main bulk of the work we have tried to assess the relative fungicidal values on a total count of not less than 1000 seedlings or ears per treatment.

The phytocidal effect of the chemicals used was recorded by germination tests carried out at the Official Seed Testing Station, Cambridge, and we here record our thanks to Mr A. Eastham and Mr C. C. Brett for their help.

In order to show the relatively ineffective nature of some of the chemicals used in this preliminary work, results that deal with the control of leaf spot of oats and bunt of wheat are recorded in Table I.

Table I. *Shows the relative fungicidal value of certain mercuric salts in the control of leaf spot of oats and bunt of wheat.*

Treatment	Leaf spot of oats. The mean of the percentage infection in five replicated plots	Bunt of wheat. The mean of the percentage infection in three replicated plots
No treatment	23	12
5 % mercuric phenate	5	5·5
10 % ,,	3	3
5 % mercurous sulphate	13	20
10 % ,,	15	19
5 % mercuric sulphate	10	12
10 % ,,	13	8
5 % mercurous nitrate	19	12
10 % ,,	20	13

Table I (*continued*).

Treatment	Leaf spot of oats. The mean of the percentage infection in five replicated plots	Bunt of wheat. The mean of the percentage infection in three replicated plots
5 % mercuric nitrate	19	12
10 % ,,	15	11
No treatment	26	10
5 % mercurous carbonate	12	18
10 % ,,	17	14
5 % mercuric carbonate	13	18
10 % ,,	10	10
5 % mercuric bicarbonate	11	6
10 % ,,	10	4
5 % mercuric sulphide	19	13
10 % ,,	24	16
5 % mercurous chloride	17	15
10 % ,,	19	16
No treatment	24	21
5 % mercuric chloride	12	5
10 % ,,	11	1·5
5 % mercuric chloride (in sol.)	14	7
5 % mercurous iodide	15	13
10 % ,,	16	8
5 % mercuric iodide	15	6
10 % ,,	5	2·8
5 % mercurous bromide	15	16
10 % ,,	13	18
No treatment	23	12
5 % mercuric bromide	14	7
10 % ,,	6	2·2
5 % mercuric arsenate	13	8
10 % ,,	11	10
5 % mercuric benzoate	14	14
10 % ,,	14	7
5 % Hydrargyri salicylas	17	14
10 % ,,	13	13
5 % mercurous oxalate	21	8
10 % ,,	17	13
No treatment	25	12
5 % mercuric oxalate	11	11
10 % ,,	10	5
5 % mercuric thiocyanate	5	4
10 % ,,	3	3
5 % mercurous acetate	17	13
10 % ,,	13	17
5 % mercuric acetate	15	8
10 % ,,	8	11
5 % amino mercuric chloride	9	7
10 % ,,	12	6
No treatment	26	9
5 % mercury	19	10
10 % ,,	16	15
5 % mercurous oxide	14	12
10 % ,,	10	15
5 % mercuric oxide (yellow)	13	7
10 % ,,	11	6
5 % red oxide of mercury	15	17
10 % ,,	13	15
No treatment	22	10

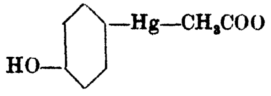
These results indicated that the majority of the inorganic compounds that we used were valueless as seed disinfectants, but they suggested the possibility of further research with mercuric phenate and mercuric thiocyanate. The following year we concentrated on the former group of compounds. These gave us results which, although not of great practical value, we thought to be of scientific interest because dressings containing this group (which are loosely described as mercuric phenate) gave in some cases a very good control of leaf spot of oats, but a mediocre or poor control of bunt of wheat.

DEVELOPMENT OF A CHEMICAL THEORY.

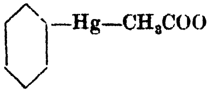
The chemistry of mercury phenate is complex and it will be considered by one of us (J. R. B.) in some subsequent study. Its chief constituents can be said to be ortho- and para-hydroxy benzene mercury hydroxide, the acetate of which was chiefly used in our trials. Whilst this compound was only moderately effective as a seed disinfectant, a very similar compound, phenyl mercury acetate, was found to be highly efficient, according to our standards at that time.

The graphical formulae of the two compounds are worthy of examination:

Para-hydroxy benzene mercury acetate



Phenyl mercury acetate



We were greatly impressed by the fact that so slight a difference in constitution should have so great an influence on fungicidal power.

It will be observed that these two compounds belong to the series *R*-Hg-*X*, and in both cases $X = -CH_3COO$, the acetate radicle. In the first case $R = -C_6H_4.OH$, and in the second $R = -C_6H_5$. These facts led to the formulation of the hypothesis that high fungicidal power is obtained when *R* is a hydrocarbon.

Field trials were put down with a wide range of compounds of the type *R*-Hg-*X* incorporated in varying percentages into dusts. The technique of these trials has already been described. The general trend of the results established the validity of the hypothesis.

The next logical step was to study the effect of variations of the hydrocarbon group *R*, and that of the acid radicle *X*.

Whilst at first sight the number of possible variants of *R* appears great, practical limitations such as stability and methods of synthesis reduce the number to the following four:

Tolyl	C_7H_7	Ethyl	C_2H_5
Phenyl	C_6H_5	Methyl	CH_3

At this stage in the investigation laboratory tests were made with vapours of pure compounds and with solutions at known concentrations. The objects of this were:

(1) To obtain evidence on the relationship of toxic action to chemical constitution.

(2) To determine the way in which these fungicides acted as seed disinfectants.

(3) To obtain information on the phytocidal effect of these salts.

The laboratory work was not extensive and we made no attempt to devise new methods to test existing seed disinfectants or to elaborate technique for a laboratory testing of fungicidal values. The method we used in the laboratory was a simple one. In our preliminary experiments watch-glasses containing Dox's medium were lightly flooded with a suspension of the spores of *Tilletia tritici* and exposed for varying periods to the vapours of the particular compounds. The amount of the spore suspension used and the periods of exposure to the vapours were constant for a particular series of experiments. Rapid drying out of the medium was retarded by superimposing watch-glasses lined with unsized and moistened paper. Later the percentage kill was determined by microscopic examination; and, subsequently, the natural fungal and bacterial contaminations that developed on the treated media were noted, and the extent of these assessed and recorded. The general trend of these experiments is indicated in Plate VIII, and this shows the extent of the contamination that developed on Dox's medium contained in watch-glasses after these were exposed for varying periods to the vapours of certain mercuric compounds. The symbols used in this diagram are P.M.Cl. (phenyl mercuric chloride), E.M.Cl. (ethyl mercuric chloride), E.M.I. (ethyl mercuric iodide), M.M.Cl. (methyl mercuric chloride) and M.M.I. (methyl mercuric iodide). The untreated dishes are not shown, but these were heavily contaminated. In the middle series in Plate VIII the lighter shading indicates bacterial and the darker fungal contamination, but this distinction is not made in the remainder and the dark shading denotes both fungal and bacterial growth.

Somewhat similar tests were made with solutions, chiefly with those containing methyl compounds. Spores of different species of *Ustilago* were used since these germinate within 24 hours, whereas those of *Tilletia* take several days. A suspension of these in distilled water was added to glasses containing either Dox or potato-agar medium, and the appropriate salt solution at a known concentration was then added. A range of salts was also tested against the organism causing blackarm of cotton (*B. malvacearum*). As a critical examination controlling environmental variables was not attempted and the tests were not extensive or many times repeated, it would serve no useful purpose to quote the fungicidal values that were obtained. The general inference was that the methyl solutions were fungicidal to the spores of *Ustilago* at very weak concentrations. and that at a given concentration they were more bactericidal to *B. malvacearum* than the corresponding ethyl compound.

Although this biological laboratory work served our purpose it was in the field that our most useful records were obtained. In this connection it is of interest to record that when spores of *Tilletia* were exposed to the vapour of ethyl mercuric iodide this appeared to exert a lethal action. When, however, a dust containing ethyl mercuric iodide was used on bunt-contaminated wheat and sown in the field there was very little control of the disease, although the same dust gave a good control of leaf spot of oats. The sodium salt of hydroxy-mercuri-dibrom-fluorescein, although a well-known bactericide, gave little or no control of leaf spot of oats. Observations such as these suggest that although some of the organic mercurials are specifics for certain seed-borne diseases, it does not necessarily follow that they are a panacea for all of them.

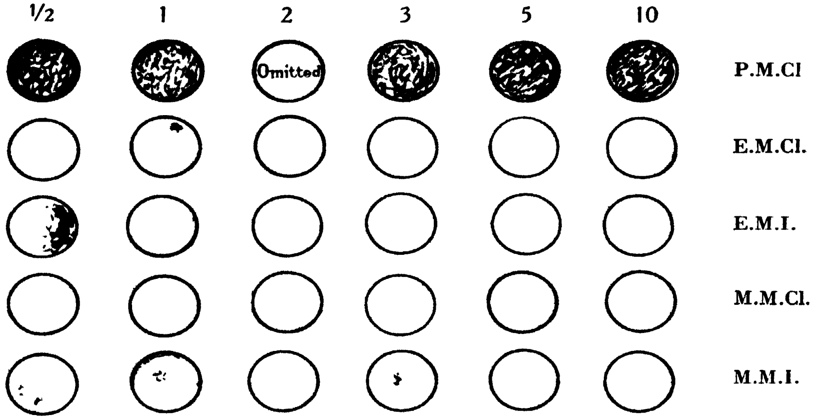
The intrinsic test in the determination of fungicidal values is the disease control that is obtained in field experiments.

The phytocidal effect of the vapours, solutions or dusts on the germination of the grain was determined in the orthodox way by submitting samples to the Official Seed Testing Station, Cambridge, and obtaining germination percentages. An example is given of the phytocidal effect of certain vapours. The pure mercury compound was spread on the bottom of a Petri dish, covered with filter paper to prevent direct contact and 400 seeds placed on it and the dish covered. The times of exposure were 6, 24, and 65 hours respectively. The normal and abnormal germination percentages are recorded in Table II.

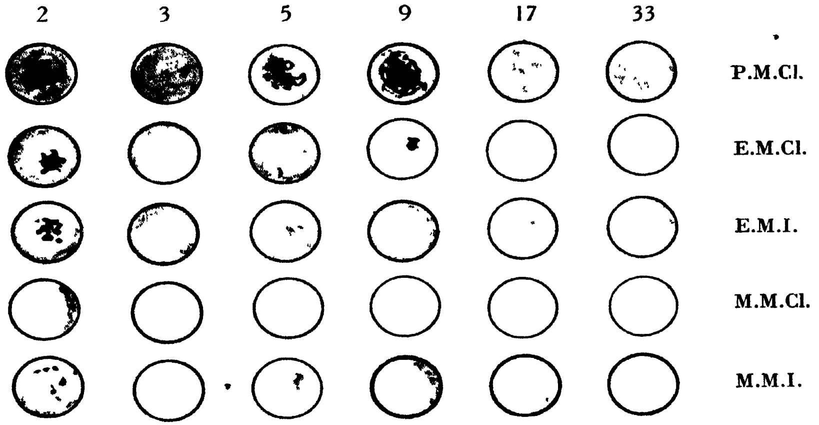
There is a similar characteristic phytocidal effect if grain is treated with a solution that is too concentrated or with a dust that contains an overdosage of the compound. This aspect is referred to subsequently.

The combined result of laboratory and field work suggested that there was a close relationship between composition and fungicidal power. In

Time of exposure in hours. Contamination recorded after 28 days



Time of exposure in minutes. Contamination recorded after 14 days



Time of exposure in seconds. Contamination recorded after 14 days

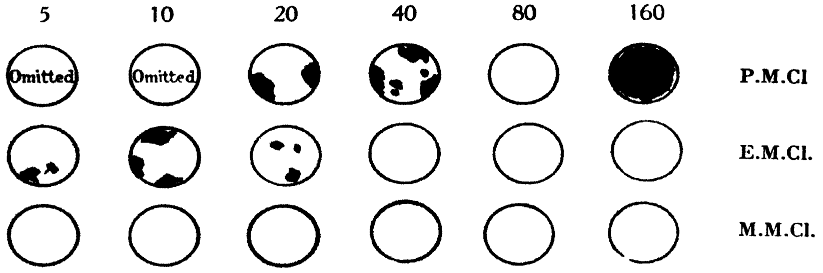


Diagram shows the extent of the natural bacterial and fungal contamination present on Dox's medium contained in watch glasses after exposure for varying periods to the vapours of certain mercuric compounds.

Table II. *Shows the normal and abnormal germination percentages of wheat when samples of this were submitted for three different periods to the vapours of certain mercuric compounds.*

Compound	Time of exposure in hours	Percentage germination	
		Normal	Abnormal
Methyl mercuric chloride	6	51	19
	24	1	18
	65	0	8
Methyl mercuric iodide	6	90	5
	24	45	29
	65	46	18
Ethyl mercuric chloride	6	99	1
	24	77	19
	65	63	30
Ethyl mercuric iodide	6	98	1
	24	96	1
	65	93	5
Phenyl mercuric chloride	6	99	0
	24	97	0
	65	98	0
Untreated seed	—	98	0
	—	99	0

the series R-Hg-X, where R is a hydrocarbon and X an acidic radicle, the fungicidal power appeared to decrease with the increase of the molecule of R. This however must not be interpreted as meaning that dusts that contain phenyl compounds are necessarily less effective in disease control than those containing ethyl or methyl compounds.

INTERPRETATION OF THE LABORATORY TESTS AND FIELD WORK.

The final stage of this particular investigation consisted of a study of the significance of X in the methyl series. The laboratory tests appeared to indicate no very marked difference in fungicidal power with the variation of X, whereas field trials had already shown that marked differences did exist under practical conditions. We have already noted that ethyl mercuric iodide gave a good control of leaf spot in oats but a poor control of bunt in wheat. It was realised, therefore, that factors such as the solubility and volatility of the compounds, and the apparent variations in the resistance of certain organisms to them, necessitated a comprehensive series of field trials if such points were to be investigated. Moreover, it was a practical advantage—as we had no evidence as to the nature of this specificity—to test these compounds against specific pathogens when they were in contact with their own hosts.

For this purpose dusts were prepared with a range of methyl mercury compounds of varying solubilities. Each compound was used from 0·1 to 1·0 per cent. in the dust in steps of 0·1 per cent. These dusts were tested for the control of the following diseases: leaf spot and loose smut of oats, leaf stripe and covered smut of barley and bunt of wheat. Contaminated or infected stocks of seed were treated at the rate of 2 oz. to the bushel of grain and the samples were dusted for the same period. The seed was sown by hand in a poor gravel soil. Each plot consisted of one rod row and for any particular treatment there were four or eight randomised replicates. The check or control plots were contaminated or infected seed that had not been treated. The disease estimates were based on a count of 250 seedlings or ears per plot—a total count of 1000 or 2000 seedlings or ears per treatment. In addition certain of these compounds were the subject of large-scale field trials at various centres in East Anglia and these were made either for observational purposes or for statistical interpretation. The results of the latter trials are not presented in this paper. The general trend of these small-scale field trials that were made to study the significance of the acidic radicle in the methyl series is shown by a series of graphs and we record our thanks here to Mr L. N. Tyrrell for the preparation of these.

Seven compounds or combinations of compounds were tested for their fungicidal value against a particular disease but it was not feasible without undue crowding to draw seven curves on one graph. Accordingly, for any one series two graphs have been made and these have been labelled A and B. Towards the base of each graph a line has been drawn at the 0·3 per cent. infection point, this we have called the "control line". The mean percentage infection for a particular disease is plotted against the percentage of compound in the disinfectant dust, and the compound or combination of compounds used is indicated. Except in the case of leaf spot of oats each major point on the curves represents the mean of the percentage infections in either eight or four replicates, in leaf spot of oats they are based on sixteen replicates. Fig. 1 A and B shows the control that was obtained for leaf spot of oats, Fig. 2 A and B gives the control for leaf stripe of barley, Fig. 3 A and B the results for loose smut of oats, and Fig. 4 A and B indicates the relative values for bunt of wheat. There is no illustration for covered smut of barley, as unfortunately the main series of experiments on this disease were abortive, as the climatic conditions at and following the date of sowing were unfavourable for infection and none resulted. It is of interest to record that the same stock of contaminated seed sown on a different date in other experiments was infected. We found that the early sown winter barley and the late sown spring barley became infected. Using methyl mercury nitrate from 0·1 to 1·0 per cent. compound in the dust and in steps of 0·1 per cent., an infection of 6 per cent. of smut in the controls was reduced in all cases to nothing when treated with dusts containing more than 0·4 per cent. of the compound. It is likely, however, that if higher infections had resulted in the untreated plots adequate control would not have resulted at such a low point.

Although it is not our purpose here to analyse these graphs in detail, it is of interest to note the effectiveness of such very small quantities of some of the compounds. If we exclude the diseases of loose smut of wheat and loose smut of barley, and consider the compounds entirely on their fungicidal values, it would seem that we have here a range of compounds that are general specifics against the common seed-borne diseases of cereals. It will be noted that methyl mercuric iodide gave a poor control of bunt in wheat. As we had previously found that ethyl mercuric iodide was also relatively ineffective in this respect, it may be mentioned here that one of us had previously worked with iodine mixed with an infusorial earth and had found that it did not control the disease.[1]

TREATMENT OF SEEDS OTHER THAN CEREALS.

During the course of this work we have made laboratory and field experiments on the disinfection of other seeds. These have included cabbage, radish, sugar beet, cotton, and field peas. In some cases promising results have been obtained and the plant population has been increased. In several of these experiments the disinfection was made with the idea of protecting the seed from the invasion of soil organisms during adverse growing weather. The work on peas was done with Mr C. C. Brett and the result of it will be given in a separate paper.

The treatment of seeds other than cereals offers we think a wide scope for investigation, and we hope to develop it. Our object here is *seed protection* (*i.e.* the protection of the seed from soil organisms) rather than *seed disinfection* (*i.e.* the killing of disease organisms on the seed).

EFFECT ON GERMINATION.

It is an obvious qualification of a seed dressing that it shall not reduce the germination of the dressed seed even on prolonged storage and this aspect of the matter has been kept under continual observation. All the

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[1] Dillon Weston, W. A. R., "Ineffective nature of iodine dust as a fungicide against *Tilletia caries*", *Phytopathology* (Sept. 1930), **20**, No. 9.

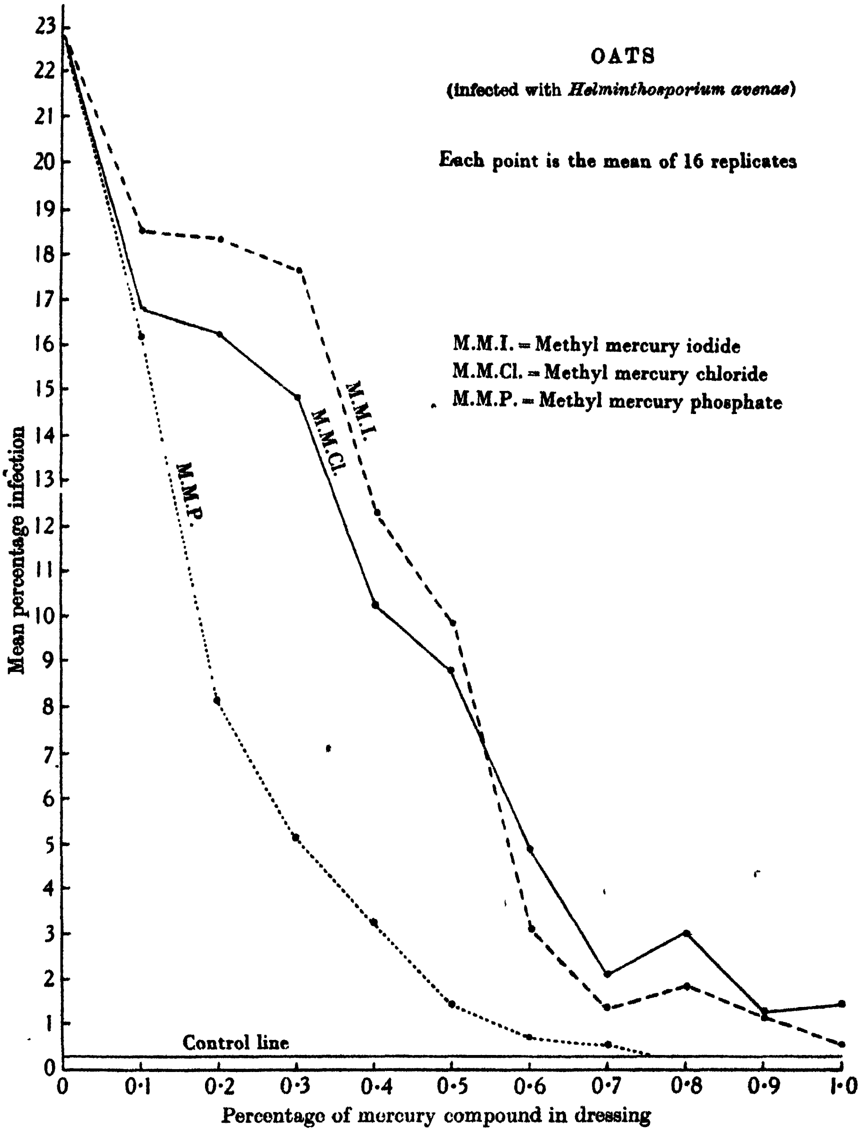
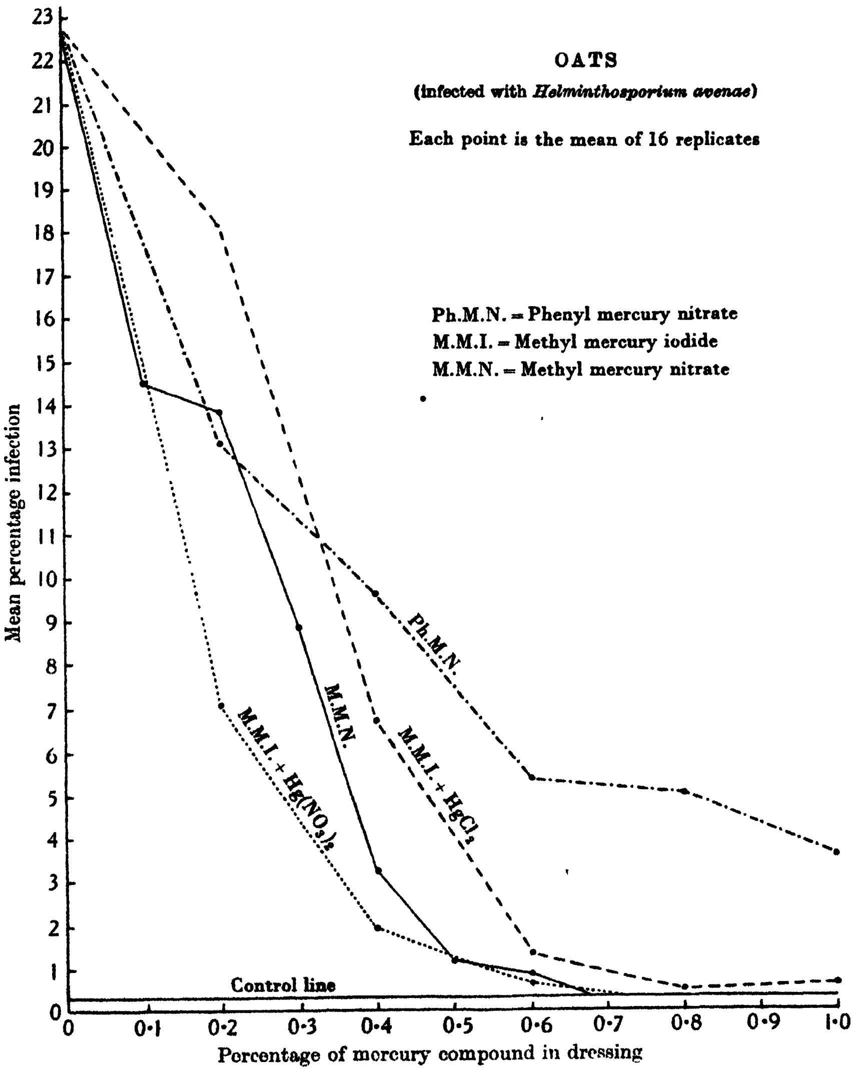

OATS
(infected with *Helminthosporium avenae*)
Each point is the mean of 16 replicates
M.M.I. = Methyl mercury iodide
M.M.Cl. = Methyl mercury chloride
M.M.P. = Methyl mercury phosphate
M.M.I.
M.M.Cl.
M.M.P.
Control line
Mean percentage infection
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 1 A.


OATS
(infected with *Helminthosporium avenae*)
Each point is the mean of 16 replicates
Ph.M.N. = Phenyl mercury nitrate
M.M.I. = Methyl mercury iodide
M.M.N. = Methyl mercury nitrate
Ph.M.N.
M.M.N.
M.M.I. + $HgCl_2$
M.M.I. + $Hg(NO_3)_2$
Control line
Mean percentage infection
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2
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0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 1 B.

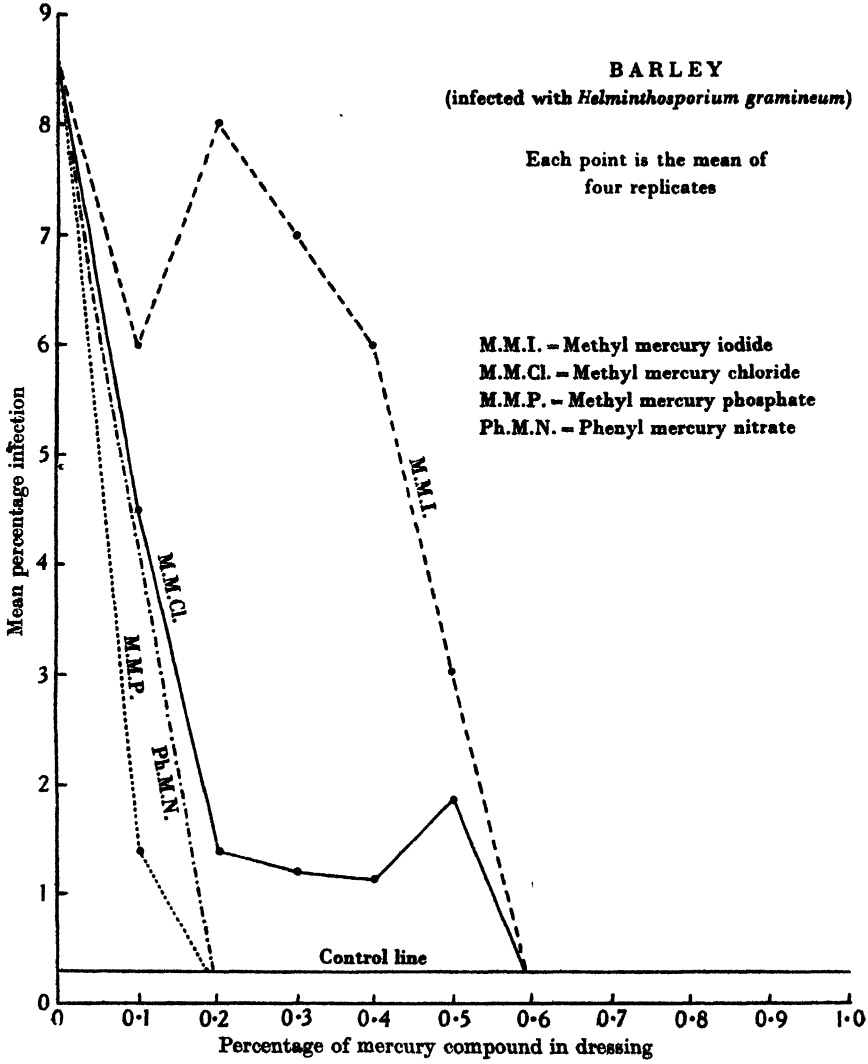
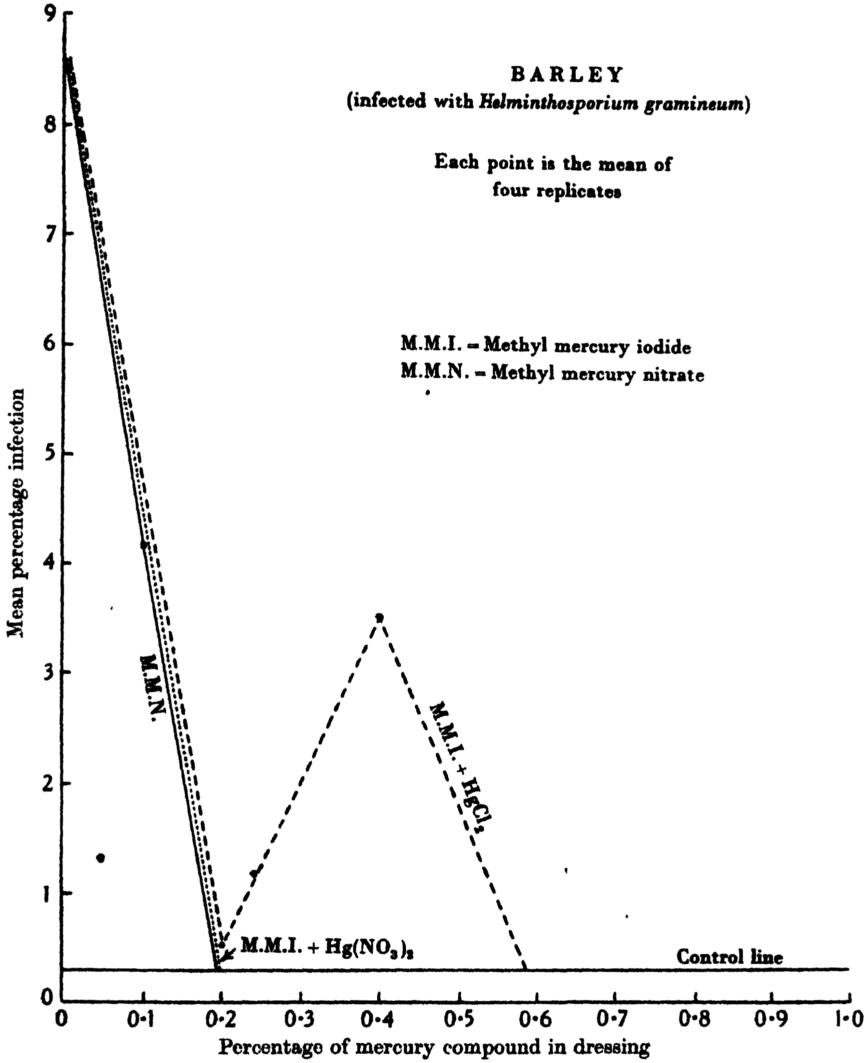

BARLEY
(infected with *Helminthosporium gramineum*)
Each point is the mean of four replicates
M.M.I. = Methyl mercury iodide
M.M.Cl. = Methyl mercury chloride
M.M.P. = Methyl mercury phosphate
Ph.M.N. = Phenyl mercury nitrate
M.M.I.
M.M.Cl.
M.M.P.
Ph.M.N.
Control line
Mean percentage infection
9
8
7
6
5
4
3
2
1
0
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 2 A.


BARLEY
(infected with *Helminthosporium gramineum*)
Each point is the mean of four replicates
M.M.I. = Methyl mercury iodide
M.M.N. = Methyl mercury nitrate
M.M.N.
M.M.I. + $HgCl_2$
M.M.I. + $Hg(NO_3)_2$
Control line
Mean percentage infection
0
1
2
3
4
5
6
7
8
9
0
0·1
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0·3
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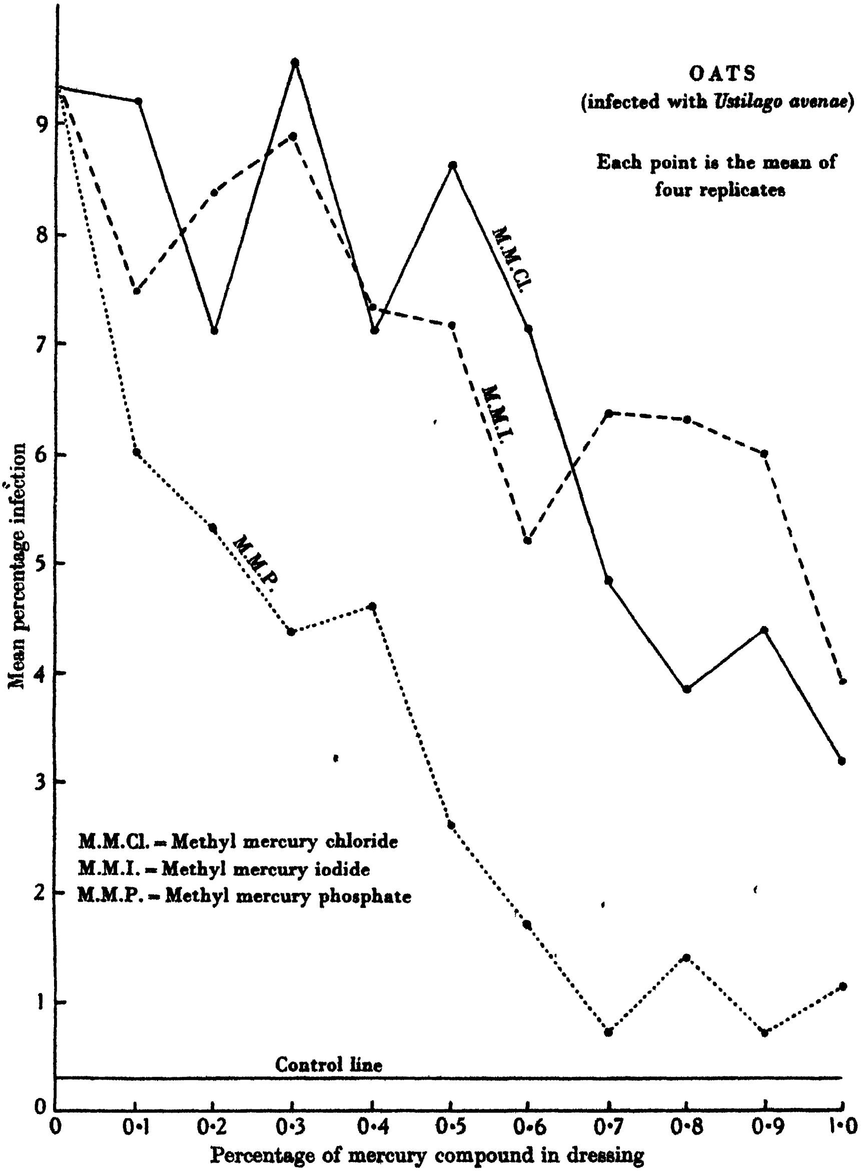
0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 2 B.


OATS
(infected with *Ustilago avenae*)
Each point is the mean of four replicates
M.M.Cl.
M.M.I.
M.M.P.
M.M.Cl. = Methyl mercury chloride
M.M.I. = Methyl mercury iodide
M.M.P. = Methyl mercury phosphate
Control line
Mean percentage infection
0
1
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3
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6
7
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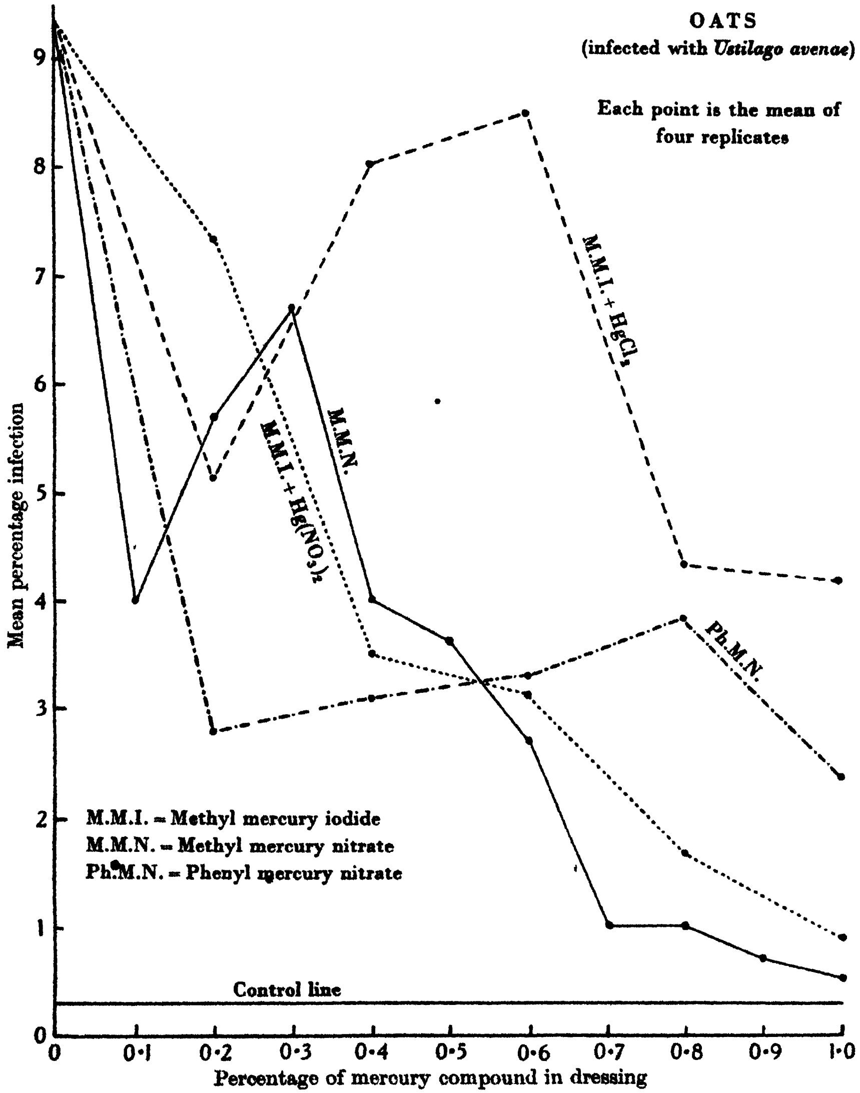
0·6
0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 3 A.


OATS
(infected with *Ustilago avenae*)
Each point is the mean of four replicates
M.M.I. + HgCl₂
M.M.N.
M.M.I. + Hg(NO₃)₂
Ph.M.N.
Control line
M.M.I. = Methyl mercury iodide
M.M.N. = Methyl mercury nitrate
Ph.M.N. = Phenyl mercury nitrate
Mean percentage infection
0
1
2
3
4
5
6
7
8
9
0
0·1
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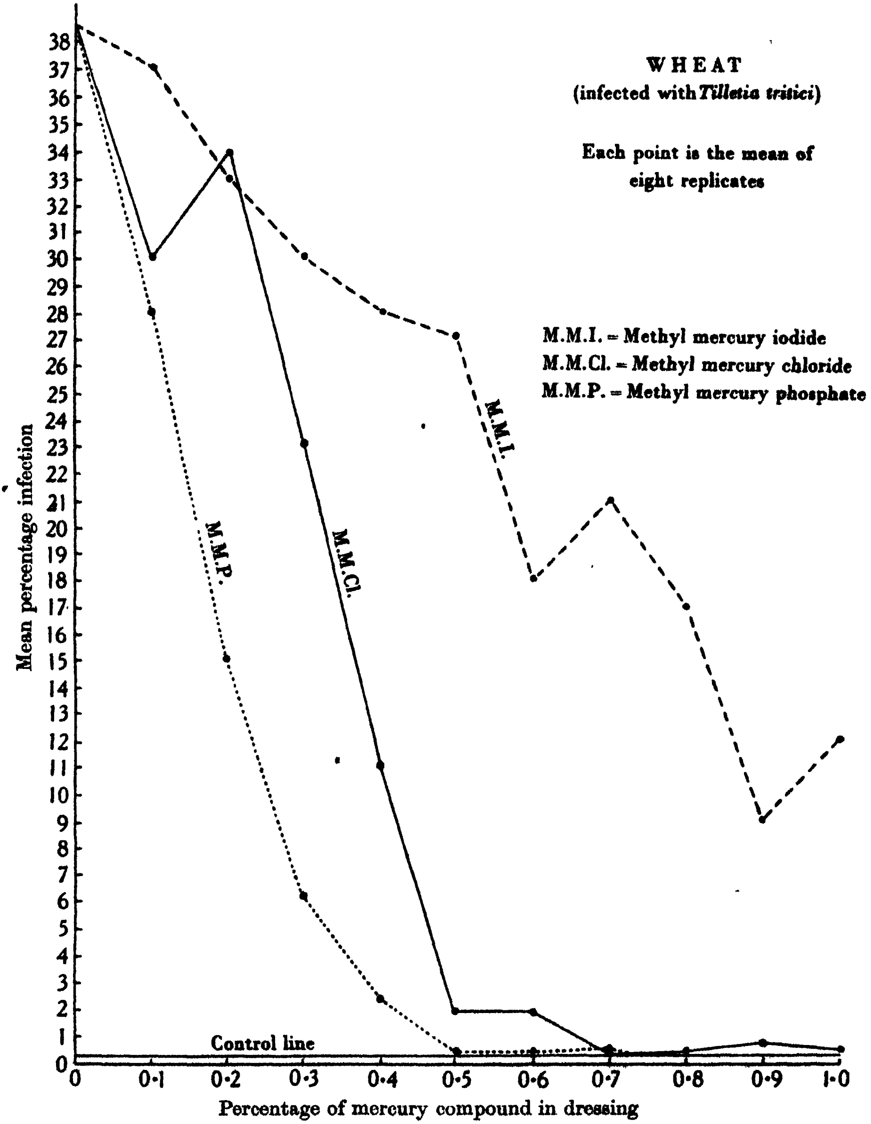
0·6
0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 3 B.


WHEAT
(infected with *Tilletia tritici*)
Each point is the mean of eight replicates
M.M.I. = Methyl mercury iodide
M.M.Cl. = Methyl mercury chloride
M.M.P. = Methyl mercury phosphate
M.M.I.
M.M.Cl.
M.M.P.
Control line
Mean percentage infection
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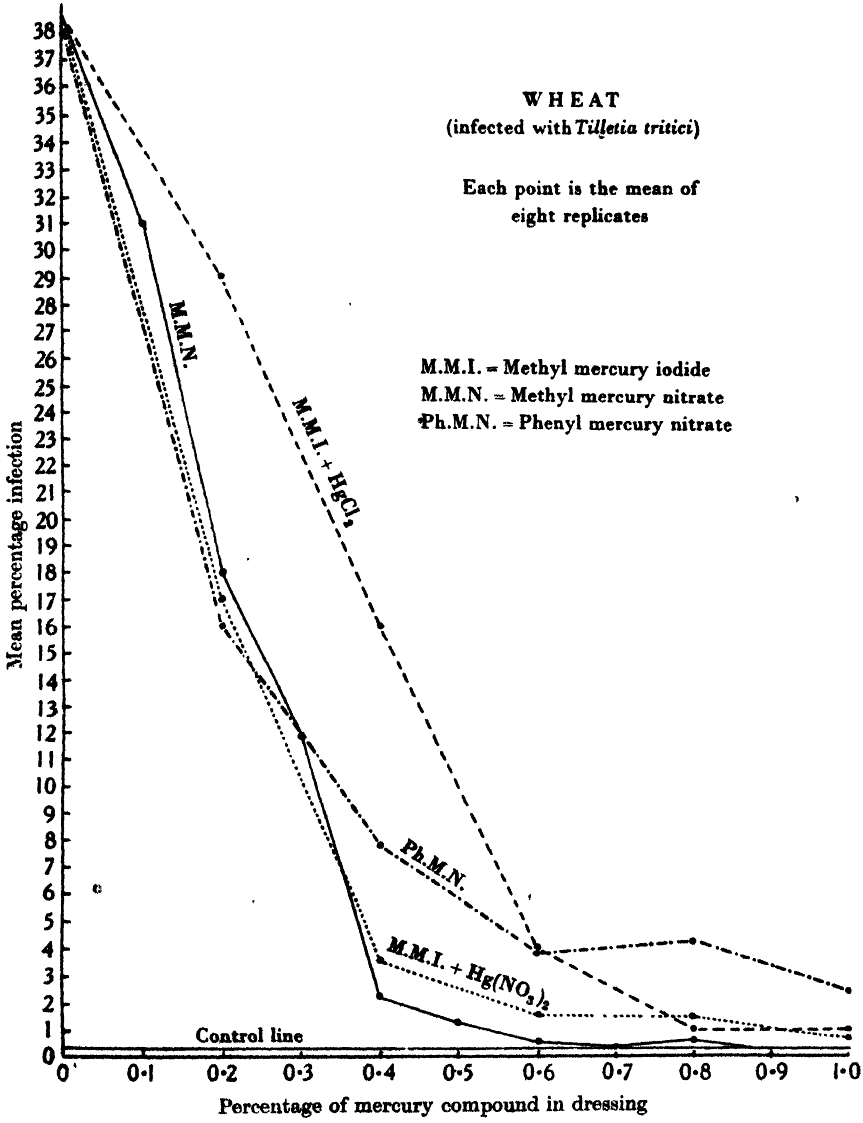
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 4 A.


WHEAT
(infected with Tilletia tritici)
Each point is the mean of eight replicates
M.M.I. = Methyl mercury iodide
M.M.N. = Methyl mercury nitrate
Ph.M.N. = Phenyl mercury nitrate
M.M.N.
M.M.I. + HgCl₂
Ph.M.N.
M.M.I. + Hg(NO₃)₂
Control line
Mean percentage infection
0
1
2
3
4
5
6
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28
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30
31
32
33
34
35
36
37
38
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Percentage of mercury compound in dressing

Fig. 4 B.

members of the series *R*-Hg-*X*, where *R* is a hydrocarbon, have a characteristic phytocidal effect when applied in overdoses and, as the symptoms have not been hitherto described, the following details may be of interest. The seed commences to germinate and the coleoptile to appear, but this develops abnormally and swells. Root development is restricted in varying degrees. These "aborted" seedlings do not develop further and do not rapidly decay. They are, in effect, mummified. Parallel with the present series of field trials, bulk samples of dressed wheat, oats, and barley stored under normal conditions have been tested at intervals and the present indications are that dusts of the methyl mercury series when applied in the correct dosages have no adverse effect on germination. The results on two bushel samples show no significant reduction after 4½ months normal storage. We have not, however, tested these samples to see if the fungicidal effect is maintained over that period of time.

STIMULATION.

Many claims of stimulation have been made for mercurial dusts but we have found no evidence of stimulation in the sense of *tonic* effect. Instances of better germination, growth, and crop yields have been observed in field trials and—as we have indicated previously—we suggest that this is due not only to the control of seed-borne diseases but to the preservation of the food reserves in the seed from the attack of soil organisms or mould organisms originally adherent to the seed. When untreated seeds are planted out on sterile media in Petri dishes the mould spores adhering to the seed very quickly germinate and soon cover the seeds and media; with treated seeds—as one would perhaps expect—this contamination is very much less.

FILLER.

In the preparation of a dust disinfectant it is necessary to add a filler for the purpose of carrying the fungicide, and it is necessary that whatever filler is used every particle must be coated with a film of the mercury compound and the method of preparing the dust must be adjusted so that this effect is achieved.

The state of subdivision of the filler must be such that the dust is adequately distributed over the seed. In the course of this investigation we have experimented with various fillers, but the filler largely used in our experiments is a non-adsorbent alumino-silicate with an average particle diameter of 9μ. It can be shown mathematically that such a filler applied to seed wheat at the rate of 2 oz. per bushel will produce a continuous layer two particles deep. There is some evidence to show that a highly adsorptive filler has an adverse effect on disease control, but this work lacks confirmation. Apart from the actual nature of the filler this must be capable of adhering to the seed or, alternatively, treated in such a way that it does so. This can be achieved by the addition of oil to the dressing and this addition also tends to allay excessive dust during application. It should be stated, however, that although work has been carried out on this factor of adhesion the use of oil in experimental dusts has been made only for one particular salt. In this case there appeared to be no interference with the fungicidal value of the mercury salt. It does not follow however that other salts will react similarly. Work on this aspect may be continued as it is at present incomplete.

TOXICITY.

Numerous vague references to the toxicity of this series of mercury compounds can be found, and although the general implication is that they are excessively toxic, such statements are not supported by experimental data. Enquiries among toxicologists revealed the fact that no accurate information was available and that no fatality had occurred—as far as was known—in England, Germany, or the United States.

It was therefore decided to obtain information and experiments were made by the Pharmaceutical Society under the direction of Prof. Burn by which it was established that toxicities to mice of this series lie between 1·0 and 1·7 times that of mercuric chloride, the methyl compounds being the most and the phenyl compounds the least toxic. The results were obtained by oral administration, and comparisons were made of the doses producing 50 per cent. mortality. We wish to record our thanks to Prof. Burn for making these experiments.

It must be remembered, however, that the dusts, as used, contain relatively small amounts of mercury and consequently any question of excessive or abnormal toxicity does not appear to arise. At the same time it must be made quite clear that these dusts are toxic and if they are used for experimentation, adequate precautions must be observed. As a bearing on this issue work has been carried out to incorporate with such dusts a dye, the object being to warn a careless operator and to make it clear that grain has been treated.

VESICANT ACTION.

An important characteristic of this series of compounds is that solutions of moderate concentrations have a powerful vesicant action. At various times during the synthesis of the pure compounds severe blistering has occurred with phenyl, ethyl and methyl compounds. No such case has, however, occurred during the preparation of the great variety of dusts which have been used in our experiments. Under stringent experimental conditions this blistering effect has been produced on the skin. In one experiment a dust containing 1·4 per cent. of methyl mercury nitrate was placed on the skin between the second and third fingers. It was moistened and bandaged for two hours. A moderately severe blister developed but there was no sepsis. It healed slowly. Other experiments of a somewhat similar nature have been carried out with other dusts and in some cases slight blistering has resulted and in other cases not. In the foregoing work, however, there are certain uncontrolled variables, such as the abrasive nature of the filler, and the percentage and nature of the oil. Our object in calling attention to the vesicant action is that under practical conditions there exists the possibility of injury to workers, and until the matter has been more fully investigated and the effect eliminated or a remedy found, efficient precautions must be taken to prevent the dust coming into contact with the skin.

It is recorded here that several of these compounds have a penetrating and disagreeable odour.

UNEXPLORED THEORETICAL ASPECTS.

Comparatively little progress can be reported on the purely theoretical aspects of the mechanism of seed disinfection. At one time it was thought that the mercury compound was distributed among the seed in the vapour phase but this hypothesis was subsequently found to be untenable. That this group of mercury compounds possesses specific fungicidal properties must be admitted, since it has been found that concentrations which are lethal to spores do not interfere with the germination of seeds; and compounds that are markedly effective in controlling certain seed-borne diseases give a poor control of others.

At present it is assumed that a solution of the mercury compound is extracted from the dust and distributed over the surface of the seed by diffusion. No information as yet can be given as to the process by which spores are killed on coming into contact with this solution.

We have done no work on the penetrative properties of these compounds, on their stability during storage, or their reaction to varying environments, but we hope to investigate such points.

Minute detail and the discussion of theoretical and practical aspects have not been attempted in this study as our main object has been to give a clear view of the scope of the investigation and its progress. At some future date we hope to publish separate studies of the various aspects of the problems that we have outlined here, and also to give the results of large-scale trials. The future work will be directed more to an investigation of the nature of fungicidal action and the specificity of some of the compounds rather than to determination of fungicidal values.

SUMMARY.

An outline is given of an investigation that has dealt with disinfectant dusts containing mercury, and it is shown that the majority of the inorganic mercury salts are of little value in this respect. Some organic mercury salts have been tested and the result of laboratory and field work suggests that there is a close relationship between composition and fungicidal power. In the series R-Hg-X, where R is a hydrocarbon and X an acidic radicle, the fungicidal power appears to decrease with the increase of the molecule of R. A study is made of the significance of X in the methyl series and the results as they apply to disease control are recorded on a series of graphs.

The specificity of some of the compounds is noted and a brief mention is made of the treatment of seeds other than cereals.

It is suggested that there is no evidence for stimulation in the sense of *tonic* effect but that instances of better germination, growth and crop yields are due not only to the control of seed-borne organisms but to the preservation of the food reserves in the seed from the attack of soil organisms or mould organisms adherent to the seed.

The phytocidal effects of the series R-Hg-X are described, their vesicant action, and relative toxicity as compared with mercuric chloride. Mention is made of the type of filler that was used.

(*Received September 4th,* 1935.)

CAMBRIDGE: PRINTED BY
WALTER LEWIS, M.A.
AT THE UNIVERSITY PRESS

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